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Transformation of healthcare models and creation of integrated care systems in an aging society: A comparative perspective of the Netherlands, Japan, and China

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SUMMARY: Faced with the global challenges of population aging and a surge in dementia cases, healthcare models worldwide are undergoing profound transformation. The Netherlands' "dementia villages" concept simulates living environments, with their antipsychotic drug usage rate (11%) being significantly lower than in traditional facilities (52%). Japan has established over 12,000 "small-scale multi-functional" care facilities, striving to achieve "life in the community." Meanwhile, China is promoting community-embedded elderly care models, exemplified by Shanghai's plan to increase the number of daycare centers from 720 in 2019 to 919 by 2024, establishing a "15-minute elderly care circle." This commentary compares the Netherlands, Japan, and China across four dimensions: aging trends, innovative care models, the development of multifunctional healthcare systems, and end-of-life care philosophies. It assesses current policy developments and practical challenges, proposing that future sustainable care systems should integrate healthcare with community resources, institutional frameworks with ethical considerations, technological advancements with humanistic values, and education on death with the preservation of life with dignity.

Keywords: aging society, dementia care, community healthcare, integrated care, deinstitutionalization, end-of-life care

1. Global trends towards an aging society

According to the United Nations' 2023 "World Population Ageing" report, the global population age 60 and over is projected to increase from 1 billion in 2020 to 2.1 billion by 2050 (*1*). More than 100 countries already have an aging society, including all high-income and middle-income nations. Approximately 60 countries are classified as deeply aging societies. One such country, Japan already has a "super-aging society," with the proportion of the population age 65 and over reaching 29.1% (2023) (*2*). The same population in China is expected to exceed 30% before 2050, with rural aging rates far surpassing those in urban areas (*3*). The pace and degree of aging vary significantly across regions: Europe and East Asia (including Japan and China) are in a deeply aging stage, while regions like Sub-Saharan Africa remain predominantly youthful, though the pace of aging is accelerating in some countries. The United States is currently in the "aged or significantly aging" stage, with 19% of its population age 65 and over in 2023, and that figure is projected to reach 25% by 2050 (*4*). (Table 1)

2. Comparison of care models: Diverging paths in the Netherlands, Japan, and China

2.1. Netherlands' "dementia villages": Centralized care in a simulated environment

"Hogeweyk," established in 2009 in a suburb of Amsterdam, is renowned as the "world's first dementia village" (*5*). By creating a familiar atmosphere through life-like settings—including supermarkets, a theater, and cafés—the project helps reduce the incidence of behavioral and psychological symptoms of dementia (BPSD). Evaluation studies have indicated that the proportion of residents using antipsychotic drugs is significantly lower than in traditional care facilities (11% *vs*. 52%) (*6*). By integrating several small "household-style units" with daily living facilities such as shops, dining areas, and public spaces into a "micro-community," the model maintains round-the-clock professional care while maximizing the preservation of daily routines and a sense of social participation among individuals with cognitive impairments (*7*). A point that should be noted, however, is that although

Table 1. Projected changes in the global population age 60 and over from 2020 to 2050

Country/Region	Population Age 65+ (2023)	Projected Proportion (2050)	Status of a Super-aged Society
Japan	29.1%	38.4%	Peaked
Netherlands	20.1%	27.6%	Steadily rising
China	15.4%	31.4%	Rapidly rising
South Korea	17.5%	38.1%	Fastest-aging country
United States	19%	25%	Transitioning from "aged" to "super-aged"
United Kingdom	17%	23%	Steadily rising

Data sources: UN DESA (2023), Japan Statistics Bureau, State Council Population Development Report (2022), "2024 National Aging Development Bulletin," "World Population Prospects 2022".

this model demonstrates significant advantages in terms of residents' quality of life, management of BPSD, and family satisfaction, it requires substantial funding, land resources, and a set team of professionals, making it difficult to replicate on a large scale in resource-limited regions.

2.2. Japan: The "small-scale multi-functional" strategy and "life in the community" strategy

Since 2006, Japan has introduced the "Small-Scale Multi-Functional Home Care" system, which integrates day service, home-visit care, and short-stay services, limiting the service scope to within 30 users to strengthen the continuity of community living and life (*8*). By 2023, there were over 12,000 such facilities nationwide (*9*). This model operates on the logic of "day-care-centered, on-demand integration of home-visit and short-stay services," typically limiting the "registered capacity" to about 30 users to ensure a small scale, frequent contact, and individualized care. Japan's Ministry of Health, Labour, and Welfare, in its Comprehensive Community Care System (2021), set the goal of "enabling elderly residents to age in place within the communities they are familiar with" (*10*). Empirical studies evaluating this model and its variants have focused on organizational operations, staffing, care practice capabilities, and the capacity to support individuals with moderate to severe needs. For example, facility surveys indicate significant variations in average manpower input and service utilization rates (*11*). Moreover, Japan has launched nationwide pilot projects for a "Dementia-inclusive Society," integrating initiatives like "Memory Cafés" and "Dementia Supporters" into citizens' daily lives (*12*). Overall, Japan's small-scale multifunctional practice offers a deinstitutionalized pathway centered on "small-scale, multifunctional integration, institutionalized payment mechanisms, and local network support." However, its effective scaling nationwide still depends on the continuous enhancement of local finances, professional human resource development, and service capacity for those with moderate to severe needs.

2.3. China: Parallel exploration of community-embedded and diversified models

In China, the State Council issued a guideline to promote the development of national undertakings for the aged and improve the elderly care system during the period of the 14th Five-year Plan (2021-2025), and it explicitly proposed creating a service system that "coordinates home, community, and institutional care and combines medical care with health maintenance," promoting "community-embedded elderly care facilities" (*13*). Community-embedded elderly care originated in Japan in the early 1980s. Similar to Japan's "Small-Scale Multi-Functional Home Care" system, China's community-embedded elderly care operates within the community, relying on one or several core facilities (such as comprehensive community elderly service centers or senior care homes) to offer various forms of specialized elderly care, providing nearby, convenient, and professional "one-stop" comprehensive elderly services for older people living at home and in the community. At its core, it is "small-scale, multi-functional, and professional," positioning it as a "third model" between institutional care and traditional family-based care (Table 2).

This model has developed with Shanghai as a prominent example. In 2019, Shanghai released the "Shanghai Community-embedded Elderly Care Work Guidelines," elevating "embedded elderly care" to a municipal-level work system and practical project. This led to a significant increase in the number of embedded facilities such as comprehensive community elderly service centers, daycare centers, and home care beds, with the radius of service availability forming a "15-minute service circle." From 2019 to 2024, Shanghai's day care centers increased from about 720 to 919, and comprehensive community elderly service centers increased from about 268 to 529. This shift indicates a reorientation of service focus towards a model "predominantly based on home and community care, characterized by a denser network of embedded service outlets," as detailed in Figure 1.

Shanghai has devised distinctive practices in care models, creating "safe gardens for exploration" on rooftops in high-density urban environments to ensure outdoor activities for people with dementia. Because of its relatively high level of population aging, Shanghai has announced policy documents such as

Table 2. Specific content of China's community-embedded elderly care model

Items	Description
Residential Scenario	Individual residence + community elderly care service facilities
Activity Scenario	Individual residence + community elderly care service facilities
Service Items	Includes professional care, meals, spiritual comfort, rehabilitation, recreational activities, *etc*.
Funding Sources	Shared by individuals, families, communities, social forces, *etc*.
Operation Models	Publicly-built and privately-operated, market operation, collective economy, charitable mutual assistance, *etc*.
Service Facilities	Comprehensive community elderly service centers, day care centers, community meal assistance points, community living stations, senior care homes, *etc*.
Main Features	Aging in one's own home, aging at one's doorstep, "services finding people", Devolution and integration of resources

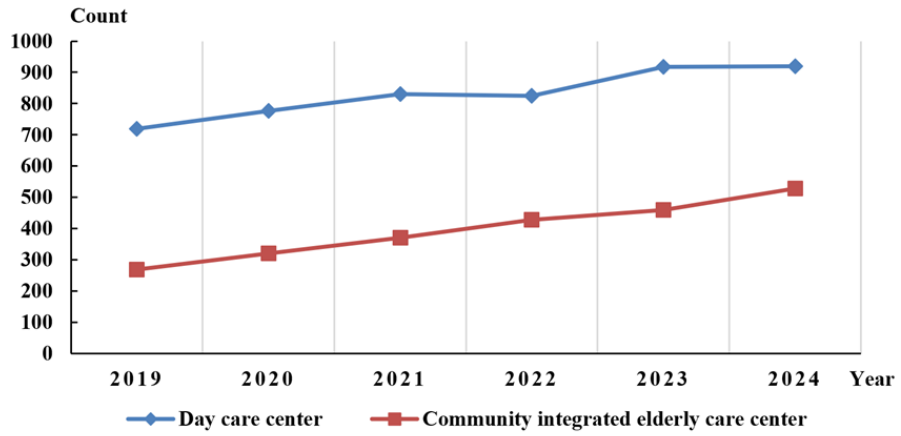

Figure 1. Number of community-embedded elderly care facilities in Shanghai (Integrated Community Elderly Service Centers, Day Care Centers). *Data Sources*: Shanghai Civil Affairs Bureau. Comprehensive Statistical Information on Shanghai's Elderly Population, Aging-related Undertakings, and Elderly Care Services. 2019-2024. *https://mzj.sh.gov.cn/2025bsmz/20250519/25e2eb0eac1e46988596a077dc4f5d8d.html* (in Chinese)

the "Shanghai Special Plan for the Layout of Elderly Care Facilities (2021-2035)," which explicitly proposes piloting rooftop gardens in multiple comprehensive elderly service centers and street-level projects, aiming to create a "15-minute community life circle." These rooftop gardens typically follow principles such as "enclosed but visible boundaries, circular and dead-end-free walkways, and wide, unobstructed, and non-slip surfaces" to balance safety and sensory rehabilitation. Chengdu created "Memory Corners in Old Teahouses" to restore cultural memories as part of reminiscence therapy. Evidence-based research has indicated that reminiscence therapy can significantly improve cognitive function, quality of life, and reduce depression and neuropsychiatric symptoms in people with dementia and cognitive impairment. In Guangzhou, municipal policies are promoting an integrated "family-community-healthcare" collaborative model centered on "Dementia Care Managers." This model features a segmented responsibility system, screening and follow-up by family doctors, and a support hub for dementia care established by the Municipal Civil Affairs Bureau, forming a layered responsibility and referral loop within the service framework.

3. Building an integrated healthcare and nursing system: Structure, challenges, and tools

3.1. Multi-functional integrated service system: A proposed core framework

The core objective of the integrated care system for a super-aged society is to achieve continuity of "person/function-centered" services, spanning the period from prevention and early intervention to end-of-life care. The proposed core architecture is as follows (Table 3).

First, primary care acts as the first point of contact and continuous management hub for the multi-functional integrated system. Its foundation should be small-scale multi-functional home care, which integrates day care, home visiting services, and short-term stays based on individual needs, maintaining a small unit size of ≤ 30 people to ensure individualized care and stable caregiver relationships. Concurrently, the family doctor contracting system should be promoted, clearly defining the primary responsibility of family doctors in screening, chronic disease and cognition monitoring, care coordination, and referral (*14*). Second, community collaboration is responsible for localizing and normalizing professional services and social resources, forming a social ecological network supporting families and the elderly. Social workers undertake case management, resource guides,

Table 3. Core framework of the multi-functional integrated service system

Component	Core Objectives	Key Elements	Representative Indicators (Examples)
Primary Services	First contact, continuous management, short-term convalescence	Small-scale multi-functional facilities (day care/home visits/short-term stays), family doctor signing	Family doctor signing rate, day care attendance, facility bed occupancy, post-discharge transfer-back rate
Community Collaboration	Integration of community resources, social participation	Social worker case management, volunteer companionship, regional support centers	Social worker-to-elderly ratio, volunteer hours, activity participation rate
Medical Coordination	Diagnosis, complex condition management, acute response	Secondary hospital dementia clinics, referral/transfer-back channels, shared EHR	Referral timeliness, 30-day readmission rate, diagnosis rate
Technology Assistance	Risk warning, expanded coverage, personalized intervention	Sensor monitoring, AI cognitive training, remote follow-up platform	Fall alarm response, remote follow-up coverage rate, changes in cognitive scale scores
Family Involvement	Sustainable care, reduced burnout, early intervention	Caregiver training, convalescent services, family education	Caregiver burden score, convalescent service utilization rate, training completion rate

and family support; volunteer networks provide companionship, daily activity assistance, and short-term convalescent care. Community-based comprehensive support centers serve as community-level hubs integrating day care, rehabilitation, cultural activities, and training resources (*15*). Third, medical linkage ensures the management of clinical complexity and timely response to acute events, serving as the medical safety net for community care. Specialized dementia clinics should be established in regional secondary hospitals or hospitals with geriatric medicine capabilities and should be responsible for diagnosis, pharmacological and non-pharmacological treatment, complication management, and discharge planning. Moreover, rapid "community-hospital-community" referral and back-referral pathways should be established, incorporating shared electronic health records and standardized post-discharge follow-up procedures (*16*). Fourth, technological assistance is an important means to expand service coverage and improve early warning and personalized intervention capabilities. The use of environmental and wearable sensors for fall prevention and activity monitoring should be promoted. Remote follow-up platforms compliant with ethical and privacy standards should be created to enable regular remote assessments by family doctors/nursing teams. Additionally, validated AI-assisted cognitive training tools should be introduced for non-pharmacological rehabilitation. The implementation of technology must address data governance, accessibility design for users with low digital literacy, and interoperability with clinical records (*17*). Finally, family involvement is core to the system's sustainability, improving the quality of care and reducing the institutional burden. Systematic caregiver training (covering communication skills, daily care, and the handling of emergencies) should be standardized and included as a reimbursable service. Convalescent care and psychological support services should be established to alleviate caregiver burnout. Early intervention education for families should be initiated at the diagnostic stage to reinforce the implementation of non-pharmacological interventions (*18*).

3.2. Challenges: Institutional fragmentation and human resource gaps

Japan is projected to face a shortage of more than 300,000 dementia care workers by 2025 (*19*), while China grapples with the rate of professional dementia care training falling below 20% (*20*), indicating a similarly severe workforce challenge. Creating the architecture mentioned earlier involves several structural challenges (Table 4).

First, institutional fragmentation and governance gaps present a major hurdle. In terms of care system governance, while the Netherlands has achieved relative integration of healthcare and care through its long-term care insurance (LTCI), significant variations in policy implementation across different municipalities have led to variations in the quality of services. Since implementing LTCI in 2000 and establishing Community Integrated Care Centers, Japan has strengthened community-level coordination to some extent, but institutional integration between health insurance, medical care, and hospice care remains insufficient. China faces the dilemma of inadequate collaboration among governing bodies, with overlapping responsibilities across healthcare, elderly care, health insurance, and civil affairs, and a lack of a unified payment and regulatory framework, resulting in a fragmented service continuum (*21*).

The second challenge is the insufficient workforce supply and little professional appeal. the Netherlands maintains a relatively robust human resource base for its long-term care workforce. However, with the surging number of dementia patients, the shortage of professional

Table 4. Challenges in building an integrated healthcare and nursing system

Challenge Category	Netherlands	Japan	China
Institutional Fragmentation and Weak Governance Coordination	LTCI as backbone, but significant municipal variations	Relatively mature LTCI, but insufficient integration between health insurance and medical care	Inadequate coordination among governance entities
Insufficient Human Resources and Little Professional Appeal	Shortage of professional caregivers, reliance on foreign workforce	Little appeal to care professionals, projected shortage of 690,000	About 500,000 professional long-term care workers, existing gap
Heavy Family Care Burden	Heavy psychological burden on family members	Approximately 100,000 people leave workforce annually due to caregiving responsibilities	Families bear the vast majority of care burden, insufficient alternative services
Lack of Coordination Mechanisms and Information Barriers	Information silos between regions	Progress through Community Comprehensive Support Centers but inadequate acute phase coordination	Lack of unified platform, weak inter-service coordination

caregivers is becoming increasingly apparent, alongside a growing reliance on foreign care workers. In Japan, the caregiving profession suffers from its image as the "3 ds" (a job that is difficult, dirty, and dangerous, often associated with low pay and status), leading to insufficient willingness among young people to enter the field. The Ministry of Health, Labour, and Welfare predicts a shortage of over 690,000 care workers by 2040. China also faces a significant care workforce gap, with only about 500,000 professional long-term care workers nationally against the needs of tens of millions of disabled older adults (*22*).

The third challenge is the heavy burden on family caregivers. The Dutch welfare system provides relatively generous coverage of family care, but for long-term care such as that for dementia, families still bear a significant emotional burden and are responsible for some daily care tasks. Japan's LTCI shares responsibility with the family to some extent, but the phenomenon of "caregiving-related job departure" remains severe, with about 100,000 people leaving the workforce annually due to caregiving. China absolutely relies on family care, with insufficient convalescent care and training systems, leaving families under significant economic, physical, and psychological pressure. Thus, although the three countries differ significantly in the degree of burden-sharing with the family, how to reduce the hidden costs and burdens of family care is a common challenge (*23*).

The fourth challenge is the lack of coordination mechanisms and information discontinuity. The Netherlands has achieved a certain degree of healthcare-care integration through municipality-led community networks, but information silos between regions persist. Japan has strengthened the connection between the community and healthcare through its Community Integrated Care Centers, yet continuity of care during the acute and end-of-life phases remains inadequate. China's coordination mechanisms are relatively weak. The family doctor contract system has not yet fully lived up to its potential, and the lack of unified case management and information platforms connecting the community, hospital, and social services often leaves patients navigating disjointed systems, resulting in insufficient continuity of care (*24*).

4. From care to "life's final chapter": Institutional ethics and cultural transformation

4.1. Deinstitutionalization and "aging in place" until the end

Recently, deinstitutionalization and "aging in place until the end" have gradually transitioned from value-based visions to practical components within the policy toolkits of various countries. The Netherlands has been implementing "aging-in-place" policies, supporting older adults to receive end-of-life care in familiar environments without moving (*25*). The core of this strategy is shifting the focus of care from large facilities to a support system centered on the community and family, institutionally making "choosing to die at home/age in place" an accessible, realistic option. Japan has also incorporated "end-of-life home care" into health insurance, with about 30% of Japanese choosing to die at home in 2022 (*26*). This reflects both institutional accessibility and is closely related to Japan's long-term promotion of a family/community care culture and investment in local medical networks. In China, the National Health Commission issued the updated Palliative Care Practice Guidelines (2025 Edition) in August 2025, further standardizing service protocols and explicitly requiring the development of core processes such as multidisciplinary team assessment mechanisms and survival estimation (*27*). Numerous countries are using policy refinement and institutional guarantees as levers to translate the "aging in place until the end" vision into practical options, all pointing to the shift of care settings from facilities to communities and families and the enhancement of accessibility to end-of-life care choices for the elderly.

4.2. Death with dignity and advance directives

To address the core ethical issue of "Who has the right to decide the end of life?", the three countries present distinctly different legal frameworks, institutional practices, and levels of social acceptance. Japan has established an Advance Directive system, yet its completion rate remains only about 10%. This indicates that even though institutional support has improved accessibility to "ending life at home," the written and operational expression of individual wishes is still not widespread (*28*). Under the Netherlands' euthanasia legislation, patient choice is broader. Since the implementation of the Termination of Life on Request and Assisted Suicide Act in 2002, the Netherlands has formed a relatively mature "legalization-review-supervision" system. The law not only allows euthanasia/physician-assisted suicide under strict conditions but also recognizes the referential value of patients' prior written wishes in specific circumstances (*29,30*). China's context is more complex. Overall, euthanasia/assisted suicide lacks nationwide legal authorization in China, and related actions may incur criminal responsibility under the current legal framework. Moreover, a unified legal status or nationwide institutional recognition for advance directives has not yet been established in China. Practice relies more on local pilot projects, hospital protocols, and academic promotion (*31,32*).

5. Conclusion: The ethical foundation and future direction of a society in which elderly live

The reform of the healthcare system is not only an update of service structures but also a shift in societal philosophy: from disease-centered to people-centered, from an institution-focused to a life-focused paradigm, from a prolongation-of-life doctrine to a dignified end-of-life experience. The future sustainable care system should be based on "Co-creating Four Conditions." First is *Co-creating a healthcare system and the community*. The close integration of a healthcare system and the community is a foundational project for responding to aging. Japan's strategy of "Living within the Community" shows that embedding care into community life scenarios can both improve service accessibility and encourage community activity. China's promotion of the "15-minute elderly care circle" needs to further strengthen the community embedding of medical resources, learning from the Dutch interdisciplinary team experience to achieve integrated prevention, diagnosis, treatment, and rehabilitation services at the community level. The future direction should be to dissolve the boundaries between medical care and life, making care a normal part of community functions. Second is *Co-creating facilities and ethics*. Institutional design must embody ethical considerations. Given the shortage of care manpower, an institutional framework that both safeguards the rights and interests of practitioners and that ensures the quality of services needs to be established. When promoting the use of technology, the ethical principle of "assistance rather than replacement" should be followed to avoid technological alienation. Third is *Co-creating technology and human connection*. Technological innovation and humanistic care need to develop synergistically. The Netherlands' reliance on technology to alleviate care shortages and China's leadership in formulating international standards for an age-friendly digital economy both highlight the potential of technology in addressing an aging society. However, Japan's experience reminds us that the use of technology must serve human needs; team collaboration and family support in its home-based palliative care cannot be completely replaced by technology. In the future, a virtuous cycle of "technology empowerment enhancing human warmth" should be created by using technology to improve efficiency, thereby allowing human connections to return to their essence. Fourth is *Co-creating education on death and life with dignity*. Education on death is an important guarantee of life with dignity. As aging progresses, there is an urgent need to incorporate education on death into the public health system, fostering a rational and calm attitude towards life through various channels such as schooling and public awareness campaigns. Only when society can face death openly can we truly achieve dignity at the end of life.

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Editorial

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Are artificial retinas merely an approach to recover sight, or are they a tool of augmented reality beyond natural eyes in blind people?

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SUMMARY: Implantable artificial retinas have been a considerable technology to help blind people recover their sight. This topic has attracted increasing attention from both patients and clinicians because of the refractory nature of degenerative retinal diseases. A point worth noting is that artificial retinas are conventionally considered to be a tool to help blind patients recover their sight. With the development of materials and sensors, however, such devices might have characteristics of augmented reality that are beyond the capabilities of the natural eye. This study briefly summarizes the current clinical status of implantable artificial retinas, it explores emerging technologies that aim to augment vision, and it discusses the challenges that must be overcome before these devices can be further used clinically. Indeed, the implantation of such advanced retinal prostheses with augmented reality characteristics may bring about new ethical and legal risks that warrant further consideration.

Keywords: degenerative retinal diseases, artificial retina, augmented reality, blind, retinal prosthesis

1. Background

Degenerative retinal diseases, such as retinitis pigmentosa and age-related macular degeneration (AMD), may cause irreversible loss of photoreceptors; however, inner retinal neurons and optic nerve pathways are commonly preserved (*1,2*). Although next-generation therapies, such as gene therapy (*3*), stem cell transplantation (*4*), and pharmacological interventions, have yielded promising results for specific etiologies, late-stage degeneration remains refractory and lacks efficient treatment. Accordingly, implantable artificial retinas, or retinal prostheses, which represent a compelling technological pathway to restore partial vision by bypassing the damaged photoreceptor layer and electrically stimulating the remaining neural circuits of the retina, have been considered (*5*). Over the last two decades, these devices have developed from laboratory prototypes to clinically tested implants, which contribute to light perception, motion detection, and basic object recognition in blind patients (*6-8*) (Table 1). However, the field is undergoing a conceptual shift: the next generation of artificial retinas may not only restore lost sight but also improve visual perception beyond the natural human spectrum with development of the computerized technology such as artificial intelligence (*5*) and sensors (*9*). This topic has attracted increasing attention from both patients and clinicians. Therefore, the current study briefly summarizes the current clinical status of implantable artificial retinas, it explores emerging technologies that aim to augment vision, and it discusses the challenges that must be overcome before these devices can be further used clinically.

2. Status of clinical study

All retinal prostheses follow a common principle, that is, the conversion of optical stimuli into electrical impulses, thereby directly activating residual retinal neurons or the optic nerve (*10*) (Figure 1A). In terms of their clinical study, current retinal prosthesis systems mainly differ in the implantation site and mechanism of stimulation as exemplified by the epiretinal Argus II (*11*), subretinal Alpha AMS/IMS (*6,8*) and PRIMA (*4,12*), and suprachoroidal 44-channel implants (*13*), the surgical accessibility, signal fidelity, and long-term stability of which are being considered in human beings. Clinical trials of these major retinal prostheses have consistently demonstrated partial restoration of light perception, object localization, and basic motion detection in patients

Table 1. Major artificial retina devices

Device/Study	Characters	Strengths	Weakness	Comments
Human				
PRIMA Holz *et al.* 2025 (*14*)	Wireless photovoltaic subretinal implant using near-IR light for activation	Self-powered; compact; achieved letter recognition under IR light	Limited field of view; requires external IR projection	Clinical phase II trial ongoing. The milestone marked the transition of artificial retina technology into the clinically applicable stage
A 44-Channel electrode Allen *et al.* 2025 (*13*)	Suprachoroidal electrode array with 44 stimulation channels for safer surgical implantation	Stable operation >2 years; minimal adverse events; low surgical risk	Limited resolution and brightness perception	Human trials ongoing. Highlights a safety-first direction in artificial retina development and provides a new pathway that balances safety and restoration of function
Alpha AMS Edwards *et al.* 2018 (*8*)	Subretinal 1600-pixel photodiode array with integrated amplifiers	Improved spatial resolution and object recognition	Requires external camera and cable link; limited field of view	Regulatory approval received. Marked a shift in subretinal prosthesis technology from experimental proof-of-concept to sustainable clinical use, breaking free of the constraints of external imaging systems.
Argus II da Cruz *et al.* 2016 (*7*)	Epiretinal 60-electrode system with external camera and processor	Partial restoration of light perception, motion, and orientation	Low visual acuity (>2 logMAR); high rehabilitation demand	Regulatory approval received. Provided valuable experience for the subsequent validation of prosthetic safety and durability
Alpha IMS Stingl *et al.* 2013 (*18*)	Early version of subretinal microphotodiode implant	Restored letter recognition and shape discrimination	Complex surgery; limited durability	Regulatory approval received, early attempts at artificial retina development
Animal				
Tellurium nanowire retinal nanoprosthesis Wang *et al.* 2025 (*9*)	Nanowire array implant converting NIR and visible light into electrical signals	Enabled both restoration of vision and infrared perception in animal models	Long-term biocompatibility under evaluation	Landmark study for "superhuman" infrared vision
POLYRETINA Vagni *et al.* 2025 (*24*)	Flexible titanium-based electrode arrays that convert light directly into stimulation currents	Achieved wireless, large-area stimulation and a wide field of view *via* foldable injectable implantation.	Only short-term safety and function verified; long-term stability unassessed.	Paved the way for the future wireless, minimally invasive, and wide-field artificial vision technologies
$AuTiO_2$-xNW arrays Yang *et al.* 2024 (*21*)	Heterostructure nanowire array with a high level of photoresponsivity	Broad spectral range; low operating voltage	Complex fabrication process	The first step in translating nanoscale photoelectric conversion into the context of primate vision
Liquid-metal-based 3D microelectrode arrays integrated with ultrathin Chung *et al.* 2023 (*25*)	Stretchable 3D liquid-metal microelectrodes for conformal neural stimulation	Excellent flexibility and durability; strong retinal adhesion	Requires further miniaturization; early preclinical stage	Promising for chronic soft implants
Plasmonic gold nanorods (AuNRs) Nie *et al.* 2022 (*28*)	Plasmonic nanoparticles that convert light into localized electric fields	High photothermal efficiency; minimally invasive	Limited control over spatial stimulation; short-term effect	Proof-of-concept stage

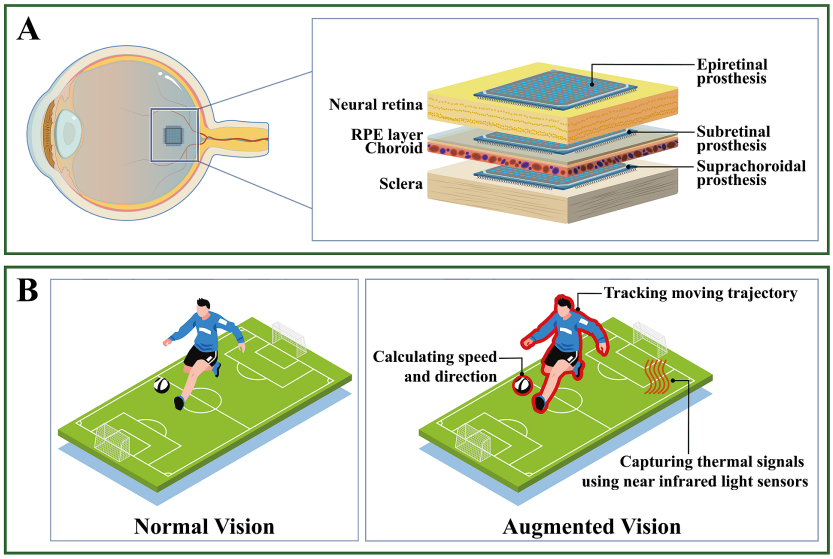

Figure 1. Diagrams of an artificial retina. (A). Diagram of the principle behind an artificial retina. **(B)**. The future of artificial retinas and the capability of augmented reality in artificial retinas beyond natural eyes, *i.e.*, the concept of "superhuman vision". RPE, retinal pigment epithelium.

with end-stage retinal degeneration, with acceptable safety and multiyear stability outcomes. Currently, several studies have documented significant progress in this field. Holz *et al*. reported that subretinal implantation of the PRIMA device in patients with AMD successfully restored central vision (*14*). Allen *et al*. demonstrated that implantation of a 44-channel suprachoroidal device did not result in severe adverse events during 2.0–2.7 years of follow-up (*13*). However, the level of restored vision remains rudimentary, and extensive rehabilitation is commonly required for functional adaptation (*15*). Nevertheless, evidence regarding safety, stability, and neural plasticity provides a solid clinical working basis for further innovation in visual prosthetics (Table 1).

Based on the technologies available thus far, development of retinal prostheses for patients still faces several interrelated challenges. The most fundamental limitation lies in the restricted number and spacing of stimulating electrodes, which spread an electrical charge within the neural tissue that diffuses the area of stimulation and results in a constraint on spatial resolution (*16*). Advances in microfabrication using novel materials such as graphene, flexible polymers, and liquid metal arrays may improve precision, but injection safety and biocompatibility must be considered (*17*). Biological integration also remains problematic; fibrotic encapsulation, gliosis, and immune responses can degrade electrode–tissue interfaces, whereas long-term implants often show increased impedance and reduced current efficiency (*18,19*). To address this issue, future designs must employ soft biomimetic materials that conform to the retinal curvature and exhibit mechanical compliance. Power delivery is another challenge because conventional trans-scleral cables or inductive coils are not satisfactory for device miniaturization and patient comfort (*20*). Novel approaches, including near-infrared (NIR) laser-driven wireless powering and photovoltaic energy harvesting, are emerging technologies for fully implantable systems that eliminate external wiring and reduce the burden of surgery (*12,21*). Finally, even when light perception is restored, patients' subjective visual experience varies widely and is impacted by the residual neural architecture, cortical adaptability, and duration of blindness. Accordingly, individualized rehabilitation, which integrates virtual reality training and neural feedback, is commonly required to enhance perceptual adaptation and optimize functional outcomes (*22*).

3. Status of preclinical studies

Emerging retinal prosthesis technologies are being developed to provide sophisticated, efficient, and biologically integrated solutions. Many novel devices or materials have been verified in preclinical studies. Photovoltaic and self-powered systems, such as the POLYRETINA implant, use titanium electrodes to convert light directly into stimulation currents, thus eliminating the need for bulky external power supplies and resulting in functional letter recognition under infrared illumination in clinical settings (*23*). A later study verified the efficacy of the POLYRETINA device in a chemically-induced blindness minipig model (*24*). Results indicated that the POLYRETINA device helped to restore light responses in blind minipigs. Chung *et al*. described a novel soft liquid-metal-based three-dimensional microelectrode that offered the advantage of reducing the impedance of the stimulation electrodes (*25*). The novel liquid-metal material showed satisfactory

proximity to the retinal ganglion cells in blind mice, thereby minimizing damage to the retina. Moreover, it can provide effective charge injections. The advantages of this novel liquid-metal-based microelectrode were verified in blind mouse models. Recently, Wang *et al*. described a tellurium-nanowire nanoprosthesis that not only restored visual function but also enabled the perception of near-infrared wavelengths, thereby extending vision beyond the normal spectral range in blind mice (*9*). These innovations have realized the dual restorative and augmentative potential of photovoltaic nanostructures. At the same time, advances in flexible and bio-integrative electronics have enhanced device-tissue compatibility. Polymer-based microelectrode arrays conform closely to the retinal curvature, improve mechanical stability, and minimize inflammatory responses (*24*), whereas stretchable optoelectronic synapses with broadband sensitivity and neuromorphic plasticity achieve a balance between optical sensing and neural computation (*26*), enabling adaptive learning prostheses that co-evolve with neural tissue. Finally, recent advances in nanotechnology offer new possibilities for retinal prosthesis implantation beyond conventional approaches. Instead of requiring the complex surgical placement of electrode arrays, tunable NIR nanoparticle sensors and plasmonic nanorods can be directly implanted into the eye *via* minimally invasive intravitreal injection, as has been done in blind rats (*27*) and mice (*28*). This less invasive and simplified surgical strategy helps to reduce procedural complexity and reduce the incidence of long-term complications. Moreover, it can offer compatibility with future electronic or optogenetic interfaces, higher spatial resolution, and lower stimulation thresholds, thus improving functional outcomes. These preclinical studies presage a bright tomorrow for implantable artificial retinas, and their future clinical applications are eagerly anticipated.

Beyond the sole aim of vision restoration, the next generation of retinal prostheses might have the potential to enhance and extend human visual perception (*26,27*). Human vision is limited to approximately 380–750 nm, where retinal photoreceptors (rods and cones) absorb light most efficiently through opsin-mediated phototransduction. Longer wavelengths carry too little photon energy to trigger the conformational changes needed for visual signaling, whereas shorter wavelengths, although energetic, are largely absorbed by the cornea and lens and can damage photopigments, thereby reducing effective vision. Emerging technologies and materials might enable artificial retinas to transcend natural limitations and evolve into a platform for sensory augmentation (*26,27*). For instance, biomimetic nanocluster photoreceptors can detect circularly polarized light, mimicking the polarization vision of crustaceans, thereby enabling improved edge detection and contrast sensitivity beyond the capabilities of the human eye (*29*). Tellurium-nanowire nanoprostheses and retinomorphic devices are sensitive to near-infrared wavelengths of approximately 980 nm, thus illustrating the feasibility of integrating infrared perception into visual systems (*10,30*). Further advances in quantum dot and perovskite materials have the potential for hyperspectral and multimodal sensing, allowing discrimination across a far broader range of wavelengths than the trichromatic human retina (*31*).

4. Concluding remarks: How to bridge the gap between the bench and bedside?

With the development of material science, neural engineering, and computational neuroscience, novel devices and suprachoroidal implants are emerging. Relying on the reconstruction of adaptive feedback loops and in-sensor processing, dynamic process stimulations, such as ambient light, gaze, or cognitive intent can be expected. In this regard, future artificial retinas can be considered not only as therapeutic tools but also as neural enhancement interfaces, offering expanded spectral awareness, adaptive contrast tuning, and context-dependent optimization (Figure 1B). At the same time, these characteristics may be beneficial for improving navigation under low-light conditions, enhancing surgical precision, and serving as experimental platforms for cognitive amelioration in a clinical setting.

However, the actual state of clinical verification thus far is that most of the clinical trials involving artificial retinas focus on recovery of primary vision. There is still a long way to go before the advanced materials and sensors mentioned earlier can be actually be used clinically. In addition to technological factors, several problems must be addressed before clinical use. *i)* An advanced assessment system that is applicable to novel artificial retinas should be devised. *ii)* A comprehensive understanding of traditional assessments, such as standardized visual performance assessments and cortical activity mapping, must be achieved to obtain comparable, unbiased, and reproducible results (*32*). *iii)* Personalized visual training and rehabilitation protocols are required. In addition, the potential for sensory enhancement in healthy individuals may pose complex societal challenges that must be addressed (*33*). *iv*) Implantation of such advanced retinal prostheses with augmented reality characteristics may bring about new ethical and legal risks. The main risk relates to the potential for "superhuman vision," with protheses that surpass medical aids and that have more functions beyond those of normal human eyes. The primary aim of implantation of artificial retinas is to help blind patients "see again," so is such "superhuman vision" really necessary for the blind? Such superhuman vision aided by artificial intelligence may lead to a "superman," but that might be considered "unfair" to normal people in whom artificial retinas have not been implanted. Indeed, the potential to have "superhuman vision" is appealing.

Thus, implantation of artificial retinas might be abused by, for example, a normal person who need not undergo that surgery. In addition, preventing such "superhuman vision" from been used for illegal purposes is also a problem. These issues require further investigation.

In conclusion, this study briefly summarized the current status of artificial retinas in terms of clinical studies, devices, and materials. Although the clinical use of artificial retinas remains limited so far, this study described promising scenarios for their use based on the development of computerized and material technologies. However, ethical issues regarding "superhuman vision" should be considered before such novel devices are actually used in clinical practice. Looking solely from the viewpoint of technology, however, such "superhuman vision" might profoundly impact not only ophthalmology but also the fields of neuroscience and cognitive science (*34*), rehabilitation (*35*) and ethics since the recovery of senses (vision, hearing, *etc*.,) helps to restore cognitive function and facilitate rehabilitation, both of which are often impacted by impaired senses (*34,35*).

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Policy Forum

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Dementia strategies in an aging society: Policies, care, and global insights from the Japanese experience

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SUMMARY: Aging of the population has become a critical challenge globally. The proportion of individuals age 60 years and older is projected to increase from 12% in 2015 to 22% by 2050, representing more than 2.1 billion older adults globally. This demographic transition is advancing particularly rapidly in Japan, which has become the first nation to become a "super-aged society". Projections indicate that by 2060, the number of older adults living with dementia will reach approximately 6.45 million (more than 17% of the elderly population), making it one of the country's most urgent health and social care challenges. Japan has developed a comprehensive response system that integrates medical, community, and family-based care. Key initiatives include a national dementia strategy, mechanisms for early screening and diagnosis, the establishment of memory clinics, and the implementation of the community-based integrated care system, which emphasizes coordination between healthcare and long-term care services. These measures have alleviated part of the burden on patients and families while enhancing social awareness of dementia and inclusion of those with that condition. Nevertheless, Japan continues to face significant structural challenges, such as severe shortages of healthcare personnel and professional caregivers, increasing fiscal pressure on long-term care financing, insufficient dissemination of innovative therapies and digital diagnostic tools, and disparities in social support between urban and rural areas. Cross-national comparisons indicate that Japan's experience offers valuable lessons for other rapidly aging societies, particularly in policy design, the integration of community-based care, and the promotion of a dementia-inclusive society. Summarizing and adapting Japan's approaches may therefore provide globally applicable strategies to build sustainable and equitable systems for dementia prevention, management, and care.

Keywords: dementia, Alzheimer's disease, super-aged society, community-based integrated care, health policy, medical challenges

1. Introduction

Aging of the population has become a serious issue globally. Aging of the population has become a serious issue globally. According to the World Health Organization Aging and health, the proportion of people age 60 years and older is projected to increase from 12% in 2015 to 22% by 2050, equating to more than 2.1 billion older adults worldwide (*1*). This demographic shift is occurring particularly rapidly in East Asia, and Japan stands at the forefront as the first country to become a "super-aged society". Currently, according to the 2025 White Paper on an Aging Society published by the Cabinet Office (*2*), the number of people age 65 and older has reached 36.24 million, accounting for 29.3% of the total population. Nearly 30% of Japan's population is age 65 or older, representing the highest proportion globally. This pronounced demographic shift has placed tremendous pressure on Japan's healthcare resources, social security system, and long-term care sector. In response, Japan has been compelled to develop optimal strategies to address population aging, thereby offering valuable insights and reference for tackling similar challenges worldwide.

Dementia is one of the most pressing health issues in aging societies, and individuals with that condition represent a particularly important population that warrants close attention. Globally, according to the

World Health Organization (WHO) (*3*), the number of people living with dementia reached approximately 57 million by 2021, with around 10 million new cases emerging each year. These figures not only highlight the rising prevalence of dementia but also underscore the substantial burden it imposes on public health and social security systems. In Japan, the situation is particularly severe: approximately 4.71 million older adults are already affected, accounting for more than 12% of the elderly population (*4*). Projections suggest that by 2060, the number of older adults living with dementia will reach approximately 6.45 million (more than 17% of the elderly population), making it one of the nation's most urgent health and social care challenges. These figures highlight a clear trajectory — without effective interventions, the burden of dementia will escalate dramatically, not only increasing morbidity and mortality but also intensifying caregiver strain, healthcare expenditures, and fiscal stress on long-term care systems. In addition, factors such as public stigma, limited awareness, insufficient financial protection, and challenges in coordinating long-term care further exacerbate disparities in the diagnosis, treatment, and management of dementia (*5-8*). These multidimensional barriers contribute to delayed care-seeking and hinder the implementation of effective community-based support systems.

Importantly, the burden of dementia is not evenly distributed across countries with different income levels, as shown in Figure 1. According to reports from the WHO (*3*) and Alzheimer's Disease International (ADI) (*9*), the most significant increase in the number of people with dementia is projected to occur in developing countries. Currently, approximately 60% of individuals with dementia reside in low- and middle-income countries (LMICs); however, this proportion is expected to rise to 71% by 2050. This rapid epidemiological transition underscores the growing global health burden of dementia, highlighting the urgent need for equitable allocation of healthcare resources, culturally tailored intervention strategies, and international collaboration to address disparities in prevention, diagnosis, and long-term care. High-income countries such as Japan, Germany, and the United States face challenges of financing long-term care systems and ensuring workforce sustainability (*10*). In contrast, LMICs, which will experience the fastest growth in older populations, face unique barriers including limited diagnostic capacity, inadequate healthcare infrastructure, and insufficient formal long-term care services (*11*). Therefore, while high-income countries face challenges in controlling costs and promoting advanced medical innovations, low-income countries must first prioritize the establishment of fundamental dementia care systems and increased public awareness. In this regard, Japan's experience — particularly its integration of community-based long-term care, development of dementia-inclusive social policies, and emphasis on early detection — offers strategies that can be adapted by other countries, including LMICs. Although resource contexts differ, the Japanese model demonstrates how coordinated policies, community mobilization, and gradual building of systems can mitigate the social and economic impact of dementia, providing lessons for countries at varying stages of demographic transition.

This paper aims to examine Japan's experience in addressing dementia, with a particular focus on long-term care. Japan has implemented comprehensive strategies, including the establishment of a national dementia plan, the promotion of early diagnosis, and the development of an integrated community care system that combines healthcare, nursing, and social support. At the same time, the country continues to face persistent challenges such as workforce shortages, financial

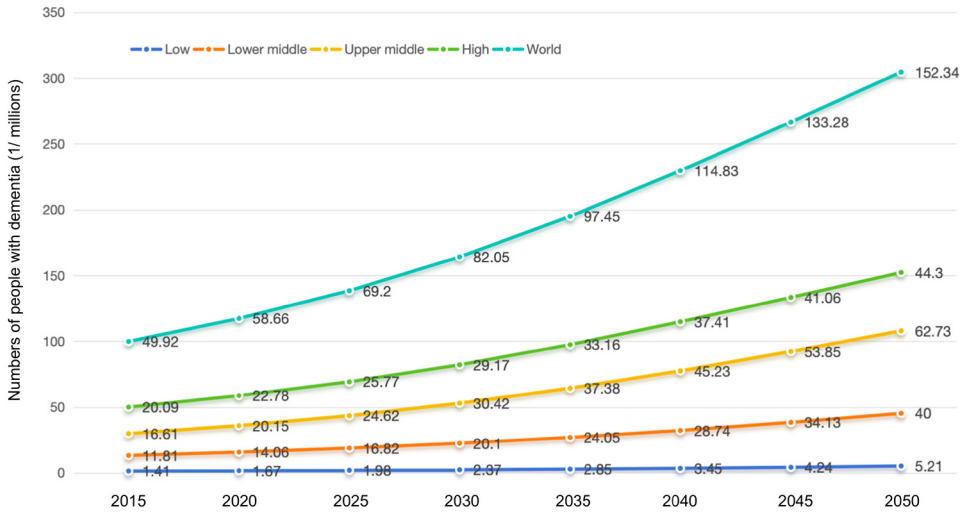

Figure 1. Estimated number of people with dementia by World Bank income group (millions) from 2015-2050. *Data source*: Alzheimer's Disease International (ADI). The number of people with dementia around the world. *https://share.google/oopaccrkijn5Ofco5*

sustainability, and inequitable access to innovative diagnostics and therapies. By analyzing the development, implementation, and limitations of Japan's policies and practices, valuable lessons can be learned by other rapidly aging societies. Ultimately, the goal is to provide global insights into building sustainable, inclusive, and patient-centered models of dementia care in the context of population aging.

2. The long-term care insurance (LTCI) system in Japan

In Asia, Japan was the first country to introduced a public LTCI system in 2000 (*12*) in response to unprecedented demographic aging, rising demand for elderly care, and the limitations of the previous tax-based and family-dominated support systems. The LTCI is a universal, mandatory social insurance scheme that covers all residents age 40 years and older through premiums, while benefits are available to those age 65 years and older who need care and to individuals age 40–64 with age-related diseases such as dementia or stroke. The LTCI provides a wide range of benefits, including home-based services (visiting nursing, day care, and convalescent care), community-based services, institutional care, and access to assistive devices. Each beneficiary undergoes a standardized eligibility assessment that determines their "level of care need", ranging from support to higher levels of dependency (Figure 2). This model has attracted international attention as a successful example of effectively addressing the challenges of a super-aged society.

Comparative studies have noted differences in care systems across income levels. In high-income countries, such as Germany and the United States, long-term care systems are often characterized by either insurance-based financing with stronger reliance on family caregiving (Germany) (*13*) or means-tested public programs combined with dependence on the private market (United States) (*14*). In contrast, middle-income countries (*e.g.*, China (*15*) and Thailand (*16*)) are still experimenting with hybrid approaches. Low-income countries, constrained by fiscal capacity, rely heavily on family caregiving with minimal institutional support, thereby amplifying unmet care needs and caregiver strain. While these systems provide important coverage, they frequently struggle with fragmented service delivery and uneven access to community-based care (*17,18*). In contrast, Japan's LTCI emphasizes universal coverage, standardized assessment of eligibility, and the integration of medical, nursing, and social services at the community level through the community-based integrated care system. This approach enables a more seamless coordination of care and reduces the overdependence on institutional settings. Notably, Japan was among the first countries in the world to explicitly identify dementia as a major focus and to incorporate it into the community-based integrated care framework within its LTCI system.

Importantly, in Japan, the majority of LTCI services are delivered through home-based care, community-based care, and facility-based care, with home-based care alone covering approximately 4.35 million LTCI recipients and facility-based care serving 0.96 million, according to data released in June 2025 (*19*). Thus, the Japanese LTCI model represents a middle pathway that balances home-based autonomy with professional support. Moreover, several studies provide empirical evidence of benefits for persons with dementia under Japan's LTCI system. Among them, "Small-scale Multifunctional In-home Care (Shotaki)" and

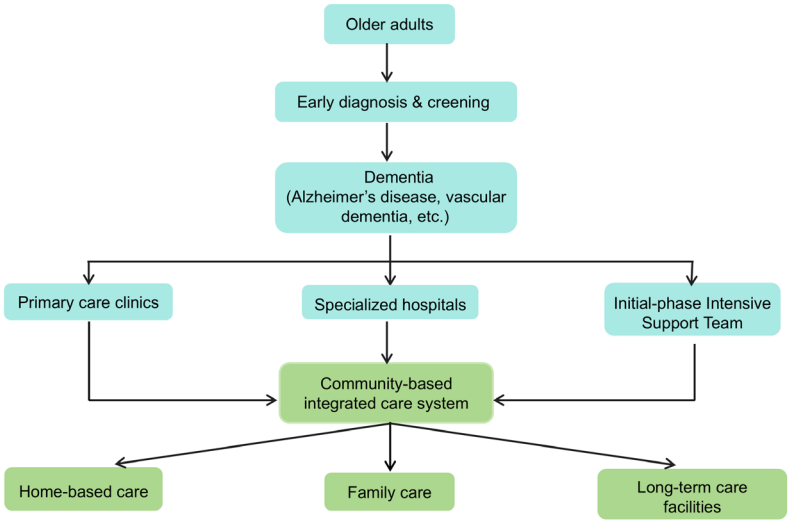

Figure 2. Schematic diagram of Japan's long-term care and dementia support system. This diagram illustrates the continuum of dementia care in Japan, from early detection and subtype diagnosis to the integration of medical, community, and long-term care services. The system emphasizes collaboration among medical facilities, community general support centers, and long-term care providers within the framework of the Long-Term Care Insurance (LTCI) system.

"Nursing Small-scale Multifunctional In-home Care (Kantaki)" have emerged as key components of the LTCI (*20*), which can also adeptly meet the demands of home care services. For instance, an observational study (*21*) found that services provided under the LTCI framework not only reduced hospital readmission rates but also addressed patients' care needs, with particularly pronounced benefits for those living with dementia. At the same time, the system plays a critical role in ensuring continuity of care. A study (*22*) found that LTCI-facilitated service provision and monitoring of care needs enabled earlier identification and timely certification of cognitive decline, providing opportunities for targeted interventions and planning care for persons with dementia. Another study (*23*) in Kyoto Prefecture found that use of LTCI services helped to reduce the level of care needs among the elderly with dementia, suggesting that these services play a protective role in maintaining functional status. In sum, the LTCI system provides a strong organizational backbone for supporting individuals with cognitive decline, though scalability and sustainability remain pressing concerns globally.

3. Policy support for dementia care in Japan: From the orange plan to the national framework

Japan has been at the forefront of formulating comprehensive national strategies to address dementia (Figure 3), reflecting the urgent challenges posed by one of the world's most rapidly aging societies. The Orange Plan (*24*), launched in 2012, represented Japan's first nationwide dementia policy, and was designed as a five-year initiative. It identified seven core pillars: *i*) creating and disseminating standard dementia care paths, *ii*) promoting early diagnosis and early intervention, *iii*) developing a community-based integrated medical care system, *iv*) developing community-based integrated medical care system, *v*) strengthening support for daily life and families in the community, *vi*) enhancing measures for juvenile dementia, and *vii*) fostering personnel for medical and nursing care. This policy has not only established a nationwide unified framework for dementia care in Japan but also provided valuable insights for the development of dementia strategies in other countries.

In 2015, the government revised and expanded the strategy into the New Orange Plan (*25*), which was explicitly framed as part of Japan's integrated community care system. Unlike the 2012 plan, which focused primarily on improving medical and nursing care, the New Orange Plan broadened the scope to include social participation, citizen engagement, and the creation of "dementia-friendly communities" This marked a shift from a strictly healthcare-oriented framework toward a more societal model that sought to empower local governments, non-governmental organizations, and civil society groups to build inclusive communities where individuals with dementia could continue to live in familiar environments. The key measures include strengthening comprehensive management across the entire disease trajectory, enhancing the training of specialized personnel, and increasing investment in research to promote the development and implementation of novel therapies. In addition, the policy emphasizes international collaboration, sharing experiences with the global community to collectively address the challenges posed by population aging and dementia.

Following Japan's implementation of the Orange Plan, several studies have evaluated its impact on end-of-life care settings, place of death, and quality of medical care for individuals with dementia. A study (*26*) analyzed nationwide death certificate data for Japanese dementia population 65 years and older between 2009 and 2016 and revealed a significant increase in deaths occurring in nursing homes and other non-hospital settings relative to hospital deaths, although home deaths did not rise

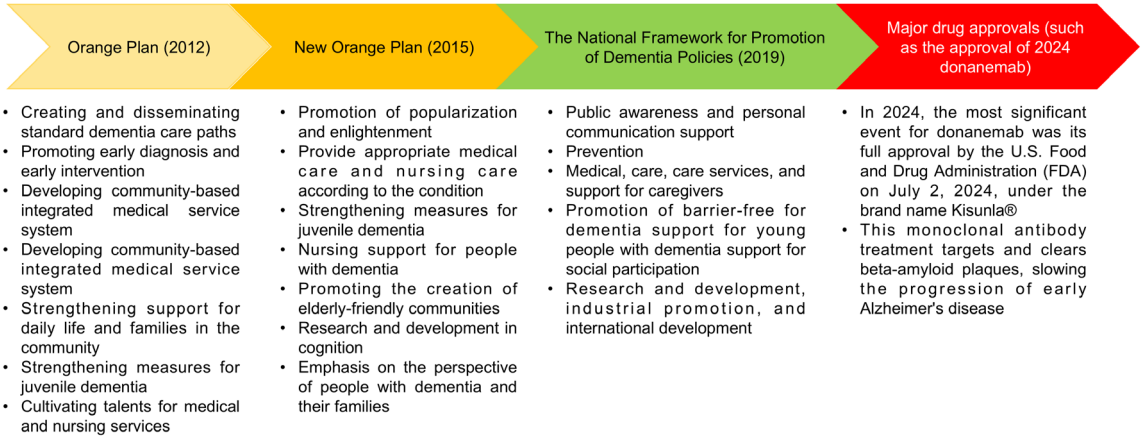

Figure 3. Dementia policy time line. *Data source*: Ministry of Health, Comprehensive strategy for the promotion of dementia measures (new orange plan): For the creation of a community friendly to the elderly with dementia (outline), *https://share.google/uA8WdAAYy8E6dPlpp.* The National Framework for Promotion of Dementia Policies (2019), *https://share.google/QNh2s581XJNKPW1q5*). (in Japanese)

appreciably. This finding suggests that the policy contributed, at least in part, to a shift in the place of death from hospitals toward institutional and community-based environments. In a retrospective cohort study (*27*) focusing on patients with moderate to severe dementia, the introduction of specialized dementia teams in acute-care hospitals was associated with a significant reduction in in-hospital mortality among those who actually received such specialized care, which indicates that specialized teams may play a beneficial role in acute-phase management for dementia patients. Nevertheless, important issues remain, including the lack of increase in home deaths, limited coverage of specialized team services, and the incomplete improvement of outcomes such as 30-day readmission rates. Although the Orange Plan and its subsequent revisions established ambitious goals and a comprehensive framework, empirical evidence regarding their effectiveness remains mixed and, at times, inconclusive. Existing studies suggest that national policies have contributed to modest shifts in patterns of healthcare utilization; however, methodological limitations preclude the attribution of these changes directly to the policies themselves. Consequently, the real-world impact of Japan's dementia policy continues to present significant challenges.

The National Framework for Promotion of Dementia Policies (*28*), approved by Cabinet decision in 2019, consolidated the previous efforts into a more permanent, long-term policy framework. Its two guiding principles were living in the community and prevention: ensuring that individuals with dementia could continue to live with dignity in their own communities, and promoting prevention and delay of disease onset through risk reduction strategies. Importantly, the Framework emphasized cross-ministerial collaboration, with the Ministry of Health, Labour, and Welfare, the Ministry of Education, and the Ministry of Economy, Trade, and Industry jointly involved in implementation. The 2019 Framework also underscored Japan's role in international collaboration, aligning with initiatives of the WHO and the G7 Global Dementia Summit, and it positioned dementia policy as a priority not only for domestic welfare but also for global health diplomacy. The same year, Japan convened a panel of experts in health policy and epidemiology to review indicators, existing research models, and emerging challenges through a series of discussions. The panel proposed methodological recommendations for evaluating both the status and outcomes of the Basic Act to Promote Dementia Policy (*29*). To date, however, Japan still lacks sufficiently mature empirical studies capable of comprehensively assessing the real-world achievement of the policy's key goals, such as building an "inclusive society" and facilitating prevention.

Overall, the trajectory from the Orange Plan (2012) to the New Orange Plan (2015) and finally the National Framework (2019) illustrates evolution from a service-oriented plan to a community-based, citizen-inclusive model and ultimately to a nationally institutionalized, cross-sectoral framework. The common threads across these policy iterations are the emphasis on community integration, early diagnosis, cross-sectoral partnerships, and sustainability. This phased approach demonstrates Japan's recognition that dementia is not only a clinical problem but a societal challenge requiring holistic, multi-faceted interventions.

4. Recent advances in drug therapy for Alzheimer's disease (AD) in Japan

In Japan, symptomatic treatments remain the mainstay for AD, such as NMDA receptor antagonists (*e.g.*, memantine) and cholinesterase inhibitors (*e.g.*, donepezil). A study of moderate-to-severe AD by Nakamura *et al.* (*30*) found that memantine has resulted in significant improvements in cognition (attention, language, visuospatial ability, and praxis) and behavioral symptoms such as agitation and aggression versus a placebo. Similarly, donepezil has been found to improve Severe Impairment Battery (SIB) and global impression (CIBIC-plus) scores in patients with severe AD, but there was no evidence of disease modification (*31*). However, the recent emergence of monoclonal antibodies has created new possibilities — and controversies — regarding the future of dementia care in Japan.

In recent years, in parallel with policy reforms, Japan has made significant strides in the approval and introduction of disease-modifying therapies (DMTs) targeting amyloid-β in AD, and particularly in its early stages. Lecanemab, a humanized monoclonal antibody targeting soluble amyloid-β protofibrils, was approved in Japan in September 2023 under the brand name Leqembi as the first DMT for early AD, which included mild cognitive impairment and mild dementia with confirmed amyloid pathology (*32*). Evidence from the CLARITY-AD phase 3 trial demonstrated that lecanemab significantly reduced clinical decline by 27% compared to a placebo at 18 months, as measured by the Clinical Dementia Rating–Sum of Boxes (CDR-SB) (*33*). Building upon this progress, donanemab, another humanized IgG1 monoclonal antibody targeting amyloid-β, received approval in Japan in September 2024 under the brand name Kisunla for the treatment of early symptomatic AD (*34*). This marked Japan as one of the earliest Asian countries to grant regulatory approval for multiple DMTs to treat AD. Evidence from the TRAILBLAZER-ALZ 2 phase 3 trial demonstrated that donanemab significantly slowed cognitive and functional decline compared to a placebo. In the Japanese subpopulation analysis, donanemab reduced decline in the Integrated AD Rating Scale (iADRS) by approximately 40% at 76 weeks among participants with a low-to-intermediate tau burden (*35*), which is consistent with global outcomes (*36*). More recently,

the TRAILBLAZER-ALZ 4 open-label head-to-head comparator trial has offered further insights by directly comparing donanemab to aducanumab in early symptomatic AD (*37*). In that study, donanemab achieved amyloid plaque clearance in 37.9%, 70.0%, and 76.8% of participants at 6, 12, and 18 months, respectively, versus 1.6%, 24.6%, and 43.1% in the aducanumab arm. Importantly, the median time to clearance was significantly shorter with donanemab, without a disproportionate increase in the risk of amyloid-related imaging abnormalities (ARIA). These findings substantiate donanemab's role as a leading DMT candidate in terms of plaque removal efficacy. However, ARIA, such as cerebral edema or effusion, were also observed among participants receiving donanemab, although most remained asymptomatic (*38*). This finding underscores the necessity of rigorous safety monitoring during treatment.

While donanemab represents a major step forward in AD management in Japan by offering a disease-modifying option beyond symptomatic therapies such as cholinesterase inhibitors and memantine, its high cost and questions regarding criteria for discontinuing treatment remain an issue. From a healthcare policy perspective, the introduction of donanemab has sparked considerable debate in Japan. The Central Social Insurance Medical Council deliberated whether and how the drug should be included in national health insurance (NHI) (*39*). The estimated annual cost of therapy, reported to be approximately 3.08 million JPY (about USD 20,000) per patient, has raised concerns regarding cost-effectiveness and sustainability, particularly in a country where the prevalence of dementia is expected to surpass 7 million cases by 2025. Critics argue that widespread coverage could strain the fiscal stability of the healthcare system, while proponents highlight the potential for delaying institutionalization and reducing long-term care costs if the drug is effectively administered to appropriate patients. Ethical considerations are also salient: determining which patients should have access, how to balance risk against benefit, and the potential for expensive therapies to exacerbate inequalities in healthcare access. In addition to pharmacological DMTs, preventive interventions are also garnering attention. Recent studies have proposed that herpes zoster vaccination may have a protective effect against dementia through mechanisms involving neuroinflammation and immune modulation (*40*), offering a complementary avenue for reducing the dementia burden at the population level.

Moreover, Japan's approval and conditional insurance coverage of donanemab stand in contrast to a more cautious stance in Europe (*41*), where the European Medicines Agency (EMA) recommends not approving donanemab for AD, positing that the benefits of this drug were not sufficient enough to outweigh the risk of potentially fatal events. In recent years, several patients have died due to microbleeds in the brain. In the United Kingdom, the National Institute for Health and Care Excellence (NICE) has rejected donanemab due to its cost and "significant health risks" (*42*). This divergence highlights the differing regulatory philosophies in countries. Japan's rapid adoption of disease-modifying therapeutic interventions not only reflects the severity of its demographic challenges but also underscores its strong political commitment to dementia policy. In summary, the approval of donanemab marks a new chapter in Japan's therapeutic landscape, but its implementation depends on a robust biomarker-based diagnostic infrastructure, equitable financing mechanisms, and ongoing pharmacovigilance. In the future, the balance between innovation and sustainability will be a decisive factor shaping Japan's dementia policy.

5. Major challenges confronting Japan

5.1. Workforce shortages

Japan's dementia policy has been lauded for its comprehensiveness, and yet its implementation faces significant human resource and fiscal constraints. The demand for dementia-related care has outpaced the supply of qualified professionals, reflecting the broader demographic imbalance of an aging society with a shrinking working-age population. According to national surveys, the number of patients requiring dementia care is projected to exceed 6.5–7.0 million by 2025 (*43*), while the pool of long-term care workers and dementia-specialized physicians is expected to lag far behind. The Ministry of Health, Labour, and Welfare has acknowledged a shortfall of more than 300,000 care workers by 2025 if current recruitment and retention trends persist (*44*). As of 2020, the care worker shortage as reported by care facility managers is as high as 60.8% (*45*). This shortage extends not only to frontline care workers but also to geriatricians, neurologists, and psychiatrists with expertise in dementia (*46,47*), creating bottlenecks in both clinical diagnosis and community-based care delivery.

Workforce shortages are compounded by high turnover rates, According to the 2024 Hospital Nurses Survey Report, the nurse turnover rate in 2023 was as high as 11.3% (*48*). Contributing factors include low wages relative to workload and significant physical and emotional strain. Studies have shown that turnover rates among care workers in Japan are consistently higher than those in other healthcare sectors, with many workers citing "burnout" and "insufficient remuneration" as their primary reasons for leaving the field (*44,49*). Therefore, the labor force shortage is an urgent problem that Japan needs to address. Although Japan has gradually opened pathways for foreign care workers under economic partnership agreements (EPA) with countries such as the Philippines, Vietnam, and Indonesia, language

barriers, interpersonal relationships, and a lack of confidence in workplace interactions have constrained their contribution (*50*). How to effectively address the growing demand for labor within the constraints of limited workforce resources will be a critical challenge in the future.

5.2. Fiscal pressures

AD dementia (ADD) costs have a significant impact on public-funded healthcare, long-term care systems, and families in Japan (*51*). A probabilistic modeling study estimated that the societal cost of dementia in Japan reached approximately 14.5 trillion yen in 2014 and is projected to increase 1.6-fold by 2060. These findings indicate that the economic burden of dementia in Japan is expected to become substantially greater in the coming decades (*52*). A more profound impact arises from the LTCI sector, where care for persons with ADD entails intensive manpower and support of a long duration, spanning at-home services, community day-care, and institutional care. In this context, dementia care has emerged as a dominant driver of growing expenditures in Japan's LTCI system (*53*). The LTCI that was introduced in 2000 provides broad coverage for community and institutional care, but the system is under financial strain due to the rising number of beneficiaries and limited revenue from premiums, which are paid predominantly by those age 40 years and older (*54*). In response, Japan has experimented with measures such as increasing copayments for higher-income older adults (*55*), promoting task-shifting to distribute responsibilities among different categories of healthcare workers (*46*), and incentivizing the use of digital technologies (*56,57*), including artificial intelligence-assisted monitoring and robotics. However, evidence on the cost-effectiveness and scalability of these solutions remains limited. The dual challenge of insufficient human resources and rising fiscal demands therefore represents a structural constraint on Japan's otherwise ambitious dementia policy framework. Addressing these shortages will require systemic reforms in workforce development, wage structures, and financing models, as well as sustained political will to balance fiscal discipline with the ethical imperative of providing equitable dementia care.

5.3. Early diagnosis and unequal distribution of medical resources

Japan's dementia policy strongly emphasizes early diagnosis, but substantial barriers persist in ensuring equitable and safe access to diagnostic and therapeutic innovations. Despite the establishment of Initial-phase Intensive Support Teams (IPISTs) and dementia-friendly consultation services under the Orange Plan, evidence suggests that many patients remain undiagnosed until the moderate or advanced stage of disease. Evidence from the City of Kobe indicates that two-thirds of patients receiving support already have moderate dementia and that over 50% wait more than one year from the first indications of dementia to interaction with the support team; moreover, only about half have a formal diagnosis of dementia at that point (*58*). The delay in early diagnosis largely stems from insufficient understanding of dementia symptoms and the disease itself among older adults, which reduces their willingness to participate in screening programs (*59*). In addition, disparities in the availability of diagnostic services — particularly in rural areas — further hinder timely detection and intervention. For example, positron emission tomography (PET) and cerebrospinal fluid (CSF) biomarker testing — necessary to confirm amyloid pathology in patients eligible for DMTs — are predominantly available in urban tertiary centers, creating a geographic inequity in diagnostic access (*60*). Japan's experience reflects a fundamental tension: while the government seeks to promote cutting-edge innovation, it must also ensure equitable and ethical distribution of diagnostic and therapeutic resources. The uneven availability of early diagnosis underscores the need for more robust evidence, including studies of real-world effectiveness and cost utility. Without such evidence, there is a risk that Japan's dementia strategy will exacerbate rather than reduce inequalities in care.

Moreover, an additional challenge lies in the implementation of subtype-specific screening and diagnostic pathways. Accurate classification of dementia subtypes (*e.g.* AD, vascular dementia, Lewy body dementia, and frontotemporal dementia) increasingly depends on biomarker and neuroimaging approaches, such as CSF and PET markers aligned with the amyloid, tau, neurodegeneration (AT[N]) framework. In Japan, multicenter PET and CSF studies have demonstrated the feasibility of AT (N) profiling in clinical settings (*61*), and yet national uptake remains limited. Meanwhile, more accessible plasma biomarkers are being actively developed. A Japanese cohort study found that the plasma Aβ42/40 ratio and p-tau217 had a high level of diagnostic accuracy in predicting amyloid positivity in PET, offering a less invasive alternative to PET/CSF that may help broaden subtype-specific diagnosis in community settings (*62*). The incorporation of plasma-based stratification could alleviate current bottlenecks in subtype diagnosis, enabling more equitable access to DMTs, assessment of eligibility, and more precise allocation of care.

6. From Japan to the rest of the globe

6.1. Three pillars: prevention, community support, and sustainable healthcare systems

The global action framework for addressing dementia is supported by three key strategic pillars: prevention of the condition and extension of healthy life expectancy,

enhanced support for communities, and the sustainability of medical and long-term care systems. The first pillar is the success of Japan's dementia prevention policy. A growing body of evidence underscores that dementia is not an inevitable consequence of aging but is significantly modifiable through targeted public health interventions. The Lancet Commission on Dementia Prevention, Intervention, and Care (2020) identified twelve modifiable risk factors — including hypertension, diabetes, smoking, hearing loss, depression, physical inactivity, social isolation, and low educational attainment — which together may account for up to 40% of global dementia cases (*63*). A review summarized (*64*) multiple studies examining the effects of lifestyle interventions — including physical activity, dietary modifications, and social engagement — on the prevention or delay of cognitive decline and dementia. The findings indicated that such interventions are particularly effective in individuals at high risk of developing dementia. For aging societies, this implies that prevention must begin decades before symptoms manifest, with policies promoting health optimization midlife and equitable access to preventive care.

Numerous studies in Japan have sought to examine measures to prevent dementia. An 18-month randomized controlled trial among Japanese older adults with MCI — which included vascular risk management, exercise, nutrition, and cognitive training — did not find a significant effect on the primary cognitive composite score overall did noted benefits in subgroups with a high level of adherence (*65*). Moreover, cohort data further corroborate the involvement of lifestyle factors; adherence to a traditional Japanese diet rich in fish, soy, vegetables, green tea, *etc.*, has been associated with a substantially reduced risk of incident dementia in long-term Japanese cohorts (*66*). Efforts addressing hearing loss also appear promising. In addition, Miyake *et al.* (*67*) found that hearing impairment was associated with mild cognitive impairment in middle-aged adults in Japan. These findings suggest that midlife hearing screening and correction may be an important aspect of dementia prevention policy.

The second pillar is the policy, which relies not only on success of Japan's dementia prevention but also on the strength of the social infrastructure that enables individuals with dementia to live with dignity within their communities. The New Orange Plan and National Framework emphasized the creation of dementia-friendly communities, so urban areas have begun experimenting with "dementia-inclusive city planning" introducing memory cafés, and volunteer-led support networks (*68*). These cafés play an important role in supporting individuals at the early stages of cognitive impairment by providing opportunities for social interaction, peer support, and private consultation in a safe and welcoming environment.

The third pillar of Japan's dementia policy framework focuses on the sustainability of medical and long-term care systems, which are under increasing strain due to population aging and the rapid rise in dementia prevalence (*69*). Japan's universal health insurance and LTCI systems, while comprehensive, face escalating fiscal pressure as the number of older adults requiring care grows and healthcare expenditures continue to rise. Ensuring sustainability requires optimizing the balance between service demand, workforce capacity, and financial resources. Recent policy directions emphasize integrated community care, the promotion of preventive health measures, and the use of digital technologies — such as artificial intelligence and remote monitoring — to reduce care burden and enhance efficiency (*70,71*). Moreover, reforms encourage task-shifting among healthcare professionals to mitigate workforce shortages and expand access to dementia services, particularly in underserved rural areas. In addition, expanding the role of nursing staff into psychosocial care, behavioral management, and adjusting the pacing of care based on patient needs has shown promise in Japan. Nurses certified in dementia nursing in acute care settings carry out assessments of apathy and tailor the frequency of interaction, the care environment, and family involvement to reduce patient distress (*72*). Training programs using virtual reality and person-centered care curricula have significantly improved nurses' confidence, attitudes, and ability to deliver both psychosocial and medical forms of dementia care (*73*). Long-term sustainability will depend not only on financial reforms but also on fostering community-based support networks and public participation to create a system that is both economically viable and socially inclusive (*74*).

6.2. Transferability of Japan's experience

While Japan has pioneered several innovative responses to dementia, the transferability of these experiences to other contexts requires careful scrutiny. Some policies are culturally embedded, while others offer universal lessons. The Japanese Integrated Community Care System (ICCS) has attracted global attention as a paradigm of decentralized, locality-driven care. Its emphasis on place-based networks — where health, welfare, and housing converge — addresses the fragmentation often seen in Western healthcare systems. Countries with comparable administrative structures and strong local governance may adopt similar frameworks; in regions with limited decentralization or weak municipal authority, however, there may be substantial barriers to implementation.

Task-shifting strategies in Japan, which empower non-physician providers and community workers, represent another potentially transferable practice. In resource-limited countries, such models can alleviate workforce shortages, particularly given the projected global shortfall of 18 million health workers by 2030 (*75*). However, successful transfer requires investment

in standardized training, supervision, and cultural adaptation. A study conducted in South Korea noted the feasibility of this program, providing further evidence of its adaptability and potential effectiveness across different cultural and healthcare settings (*76*). Japan's digital innovations in AI-based cognitive screening may offer some of the most universally applicable insights. Japan has developed a computer-based cognitive assessment tool designed for the early detection of dementia risk (*77*). Such systems have the potential to transcend cultural boundaries and may be particularly valuable in regions with dispersed populations. However, challenges related to digital literacy, privacy, and health data governance must be addressed to ensure their global scalability and ethical implementation.

6.3. Japan's policy paths from the short term to the long term

Addressing the global dementia challenge requires a phased strategy that balances immediate feasibility with long-term sustainability. Short-term policies should focus on strengthening early diagnosis and risk reduction. Governments can integrate dementia risk assessments into existing non-communicable disease (NCD) prevention programs, promote public awareness campaigns regarding modifiable risk factors, and expand training programs for general practitioners to recognize early cognitive decline. Medium-term strategies should emphasize system-building and capacity expansion. This includes developing community-based care infrastructures modeled on Japan's integrated approach, scaling up task-shifting to alleviate human resource shortages, and investing in caregiver support systems. Fiscal reforms, such as outcome-based reimbursement for new therapies and targeted subsidies for high-need groups, can help manage rising healthcare expenditures. In parallel, the deployment of digital tools for screening, monitoring, and caregiver communication should be expanded to bridge gaps between urban and rural areas.

Long-term policies must envision sustainable systems that can endure demographic pressures. This requires embedding dementia-friendly principles into urban planning, ensuring accessible public transportation, housing modifications, and inclusive community spaces. At the financing level, governments should pursue multi-payer models that balance public insurance with private-sector innovation while protecting equity. Priority should be given to research investments in novel therapeutics, preventive vaccines, and social intervention strategies, accompanied by global collaboration to pool data and accelerate discovery. Moreover, international coordination — through WHO or regional consortia — can facilitate knowledge transfer and harmonized strategies across nations at different stages of demographic transition. Ultimately, an effective global dementia strategy must align public health imperatives with social values, ensuring that aging societies remain not only medically supported but socially inclusive. By combining evidence-based prevention, robust community support, and sustainable financing, policymakers can formulate dementia strategies resilient enough to withstand the profound demographic shifts of the 21st century.

7. Conclusions and Perspectives

Japan's experience offers valuable insights into the governance, policy, and care frameworks required to respond to dementia in super-aged societies. However, it also highlights the complexities of balancing innovation, sustainability, and equity in dementia care. To address the growing global burden of dementia in a scientifically rigorous and socially equitable manner, coordinated international action is essential. Such efforts should integrate dementia prevention, community-based support, and sustainable healthcare systems into a cohesive global framework. This approach not only promotes health equity and cost-effectiveness but also ensures that individuals living with dementia can maintain their dignity and quality of life across diverse cultural and socioeconomic contexts.

Several key lessons can be learned. First, prevention must remain central, supported by evidence from the Lancet Commission and WHO guidelines showing that up to 40% of dementia cases may be attributable to modifiable risk factors (*63*). Second, sustainable dementia strategies must extend beyond the biomedical domain to encompass community-based support, caregiver protections, and dementia-friendly urban design. Third, the Japanese model reveals both transferable practices — such as integrated community care, task-shifting, and digital screening—and culture-specific elements that may require adaptation in other contexts. For countries at earlier stages of demographic transition, these insights offer a roadmap for proactive policy design.

Nonetheless, significant challenges persist. Japan continues to face shortages of specialized physicians and long-term care workers, highlighting the fragility of workforce sustainability. Fiscal pressures are intensifying, particularly as new costly therapies enter the market. Moreover, early diagnosis remains uneven, with rural-urban disparities in resource availability. Ethical debates surrounding DMTs — and especially in light of uncertain clinical benefit, risks of amyloid-related imaging abnormalities, and inequities in reimbursement — further hamper implementation. These limitations underscore the need for cautious, evidence-based decision-making rather than uncritical adoption of novel interventions.

This review also has its own limitations. Much of the existing literature on the effectiveness of Japan's dementia policies, such as evaluations of the Orange

Plan, remains preliminary and descriptive rather than based on rigorous long-term outcomes or cost-effectiveness analyses. Evidence gaps also exist regarding the scalability of integrated community care outside Japan, the long-term impact of digital screening tools, and the comparative effectiveness of emerging therapeutics in real-world settings. Moreover, Japan provides an illustrative case, but extrapolation to countries with different cultural, institutional, or economic contexts must be done carefully.

Future research should therefore prioritize three directions. First, cross-country pilot studies are needed to evaluate the feasibility of transferring elements of the Japanese model, and particularly integrated care and community-based interventions, to diverse healthcare contexts. Second, robust economic evaluations — including cost-utility analyses of DMTs and community-based prevention — are essential to guiding resource allocation in both high-income and resource-limited settings. Third, long-term cohort studies and real-world follow-ups are critical to assessing the sustainment of therapeutic benefits, evolution of the caregiver burden, and the broader societal impact of dementia strategies.

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Dual community-based care innovations in a super-aged population: The role of Small-scale Multifunctional In-home Care and Nursing Small-scale Multifunctional In-home Care in Japan

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SUMMARY: With the accelerating trend of population aging, Japan has become the first country to enter a "super-aged society", where the proportion of people age 65 and over exceeds 21%, making it a global model in addressing aging-related challenges. In response to the various social and healthcare issues arising from this demographic shift, the Japanese Government has implemented a series of policy measures. Among them, "Small-scale Multifunctional In-home Care (Shotaki)" and "Nursing Small-scale Multifunctional In-home Care (Kantaki)" have emerged as key components of the community-based care system. This paper explores the common challenges faced in super-aged populations and provides a comparative analysis of the functions, current status, existing issues, and future prospects of "Shotaki" and "Kantaki". By examining these two service models, the study aims to offer policy recommendations and practical insights to build a sustainable elderly care system.

Keywords: long-term care insurance, community-based integrated care, population aging policy, innovations in service delivery, home- and community-based services (HCBS)

1. Introduction

Japan became the world's first country to have a "super-aged population" in 2007, when the proportion of people aged 65 and over exceeded 21%. Since then, population aging has continued to intensify. According to the 2025 White Paper on an Aging Society, as of October 1, 2024, Japan had 36.24 million people age 65 and over, accounting for 29.3% of the total population (*1*). Of those, people age 75 and over accounted for 16.8%. By 2025, the entire baby boomer generation will have reached the age of 75 and over, causing what is commonly referred to as the "2025 Problem (*2*)". Moreover, as of March 2022, 6.9 million individuals had been certified as requiring long-term care or support, reflecting a 3.2-fold increase from the level in 2000 (*3*). In response to these significant demographic shifts, Japan has been actively promoting the establishment of a Community-based Integrated Care System and has expanded various forms of in-home support services (*4*).

Since the introduction of the Long-term Care Insurance system in Japan in 2000 (*5*), various forms of care services have been developed, including home visits, outreach services, and institutional care. In 2006, with the revision of the Long-term Care Insurance Act, the Small-scale Multifunctional In-home Care service was established. Targeting groups of fewer than 29 individuals, this model integrates day care, short-term stays, and home visits into a comprehensive 24-hour service centered on "frequent community-based interaction", aiming to support older adults in continuing to live at home (*6*). However, the original design of the system focused on providing daily living support rather than medical care, which has led to certain limitations in accommodating older adults with high medical dependency.

In response to the increasing severity of care needs and the growing demand for medical support, Japan institutionalized the Nursing Small-scale Multifunctional In-home Care service (formerly known as "comprehensive services") in 2012 (*7*). By integrating home-visit nursing, this service has been positioned as a community-intensive care model, capable of accommodating patients with higher levels of medical dependency. Currently, both "Small-scale Multifunctional In-home Care (Shotaki)"

and "Nursing Small-scale Multifunctional In-home Care (Kantaki)" are increasingly recognized as core pillars of Japan's community-based long-term care system. This paper explores the common challenges faced in super-aged populations and provides a comparative analysis of the functions, current status, existing issues, and future prospects of "Shotaki" and "Kantaki". By examining these two service models, the study aims to offer policy recommendations and practical insights to build a sustainable elderly care system.

2. Key issues in a super-aged population

2.1. Rising national long-term care expenditures and increasing demand for at-home services and the growing demand for living at home

According to the 2023 Survey on the Actual Status of Long-Term Care Benefits by the Ministry of Health, Labour, and Welfare of Japan (*8*), the total number of individuals receiving preventive and long-term care services reached 67.08 million, representing a 1.9% increase compared to 2022. Of these, 10.81 million received preventive care services, while 56.29 million utilized long-term care services. These figures underscore the growing demand for care services among the elderly population. Correspondingly, national expenditures on long-term care benefits have continued to rise, representing a 2.9% increase compared to 2022. In terms of service types, notable increases in the number of service recipients were observed in at-home services (up 2.0%), home care support (up 1.2%), and community-based intensive services (up 1.5%), all of which exceeded the growth in facility-based services (up 0.6%). This trend indicates a growing preference among older adults for receiving care in familiar home environments rather than institutional settings, highlighting the increasing need for at-home care services.

2.2. Changes in family structure

Japan's family structure has undergone significant changes in recent decades, with a marked increase in the number of elderly people living alone. As of 2023, households with at least one member age 65 or older accounted for 49.5% of all households (*9*). Nearly one-third consisted solely of elderly couples or individuals living alone. This demographic shift has raised concerns about the physical, emotional, and social support needs of older adults living independently. At the same time, there is a growing preference among the elderly to spend their final days at home. According to the 2017 national survey on end-of-life medical care preferences, over 43.8% of respondents expressed a desire to die at home (*10*). This trend reflects an increasing inclination among older adults to seek comfort and dignity in familiar surroundings rather than in institutional settings. Consequently, establishing sustainable, individualized home- and community-based care systems has become a critical challenge for healthcare and social support services. Addressing this issue is essential to achieving the goal of "aging in place" and ensuring quality of life in the final stages of life.

2.3. Rising mortality and the urgent challenge of end-of-life care

Japan is entering an era when it is often referred to as having "a population with an exceedingly high mortality", characterized by a continuous rise in annual deaths. In 2024, the number of deaths reached 1,605,298, an increase of 29,282 compared to the previous year. The mortality rate also rose from 13.0% in 2023 to 13.3% (*11*). The number of deaths is projected to increase to 1.6 million by 2030, peaking at 1.68 million in 2040 (*12*). This demographic trend underscores not only the accelerating aging of the population but also the growing demand for end-of-life and palliative care services. As shown in Figure 1, the number of Nursing Small-scale Multifunctional In-home Care (Kantaki) facilities has also been increasing year by year. However, ensuring appropriate and dignified end-of-life care for a growing elderly population presents substantial challenges. Hori *et al.* (*13*) warned that providing adequate care for all elderly individuals in their final stage of life will become increasingly difficult. Following the issue of so-called "care refugees (*14*)" — elderly individuals unable to access necessary long-term care — concerns have now shifted toward the emergence of "palliative care refugees", referring to those unable to receive proper end-of-life care. Japan now faces a dual crisis: the urgent need to reform care systems for frail older adults in a super-aged population with a declining birthrate, and the development of a comprehensive and inclusive framework for end-of-life care in the face of rising mortality. Addressing these challenges will require coordinated efforts among government agencies, healthcare facilities, and community-based services to ensure that all individuals can experience a peaceful, dignified, and well-supported end of life.

2.4. The increase in the number of patients with Alzheimer's disease (AD)

AD is a neurodegenerative disorder that affects millions worldwide and is expected to surge in prevalence due to aging populations (*15*). As Japan's population becomes even more super-aged, the number of people living with dementia has been steadily increasing. According to the Ministry of Health, Labour, and Welfare (*16*), as of 2022, approximately 4.43 million individuals age 65 and older had dementia, accounting for 12.3% of the elderly population. By 2060, this number is expected to exceed 6 million, accounting for more than 17% of the elderly

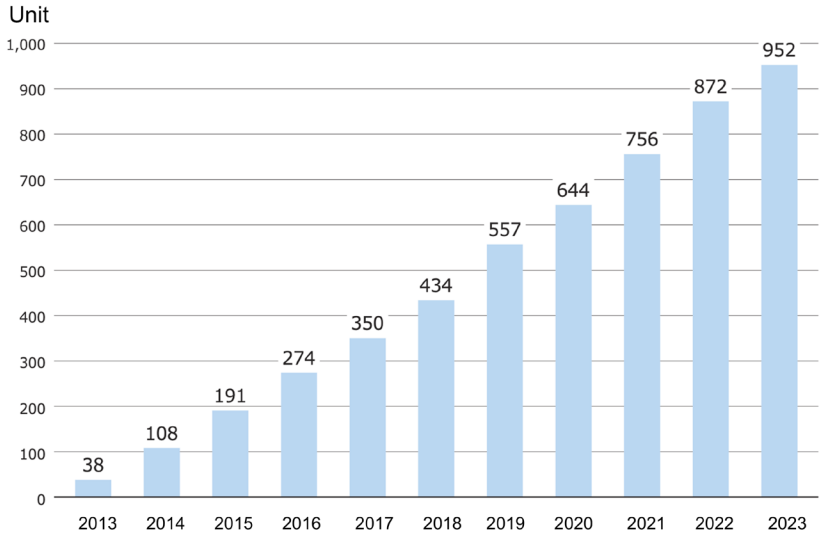

Figure 1. Trends in the number of Nursing Small-scale Multifunctional In-home Care (Kantaki) facilities. (*Data Source*: Ministry of Health, Labour, and Welfare. *https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000091038.html*).

population and posing a substantial challenge to the country's long-term care system. Against the backdrop of policy initiatives promoting "an inclusive society" (*17*), "Shotaki" and "Kantaki" have been positioned as key service models to facilitate community-based living for people with dementia. Although these care modalities have helped to facilitate living at home, significant limitations remain in terms of service coverage, professional capacity, and responsiveness to acute-phase needs. Facilities are disproportionately concentrated in urban areas, leaving rural and depopulated regions underserved. Moreover, "Shotaki" lacks the medical and nursing infrastructure necessary to manage the complex care requirements of individuals in moderate to severe stages of dementia — particularly in the management of behavioral and psychological symptoms of dementia (BPSD), medical decision-making, and end-of-life support. While "Kantaki" integrates nursing services, it frequently suffers from workforce shortages that hinder the delivery of high-intensity, continuous care (*18*). These gaps highlight the need for systemic reforms and strategic allocation of resources to meet the growing and diversified care demands of dementia patients.

2.5. The prevalence of severe frailty among the elderly

The influence of frailty on the health of the elderly has been a hot topic in recent years. As a dynamic and reversible geriatric syndrome, it has become one of the important public health problems emerging around the world (*19*). Based on a survey utilizing nursing care data, the health status of older adults across different age groups was examined, with a focus on identifying the major disease-related causes of health deterioration. The results indicated that, among the late-stage elderly population age 75 and over, the leading cause of requiring long-term care in 2019 was dementia, accounting for 22.2% of cases. This was followed by age-related frailty, which constituted 16.5% (*20*). Frailty is a geriatric syndrome characterized by a multisystem physiological decline, increased vulnerability to stressors, and adverse clinical outcomes (*21*), which has led to a greater reliance on life care services. In terms of policy, "Shotaki" and "Kantaki" are considered particularly suitable for providing individualized and continuous support and health management for frail older adults. This facilitates the early detection of frailty symptoms, health education, and preventive interventions. In practice, however, "Shotaki" and "Kantaki" often fall short of meeting the complex needs of the frail elderly population. Moreover, there is a lack of systematic assessment tools (*22*) and evidence-based intervention guidelines for frailty (*23*), hampering the implementation of effective early-stage management.

3. Current Status of "Shotaki" and "Kantaki"

Differences between "Shotaki" and "Kantaki" are summarized in Table 1. As of April 2023, data from Japan's Ministry of Health, Labour, and Welfare showed that there were 994 "Kantaki" facilities nationwide, a considerably smaller number compared to 5,523 "Shotaki" facilities (*24*). The expansion of "Kantaki" facilities in urban areas in particular has been hindered by challenges such as attracting qualified medical personnel and concerns over operational profitability, as shown in Figure 2. A nationwide survey conducted in 2020 revealed that the number of "Kantaki" facilities was positively correlated with the number of "Shotaki" facilities, visiting nursing care providers, the total population, and population density but negatively correlated with the regional aging rate (*25*). This suggests that "Kantaki" facilities are more likely to be established

Table 1. The differences between "Small-scale Multifunctional In-home Care (Shotaki)" facilities and "Nursing Small-scale Multifunctional In-home Care (Kantaki)" facilities

Variable	"Shotaki" facilities	"Kantaki" facilities
Services provided	Daytime services, care, short-term accommodation	In addition to the aforementioned services, home care services are also provided
Participants	The elderly population with moderate-level care needs	Elderly people requiring medical care (such as intravenous injections, suctioning, and hospice care)
Medical treatment	Medical treatment is limited, so home nurses are provided through external cooperation.	There is always a nurse on-site who can provide immediate medical treatment.
Nurse allocation	Not necessary (can cooperate with external visiting nurses)	Mandatory deployment of at least 2.5 full-time nursing staff
Legal basis	Long-term Care Insurance Act (regional services)	Same as above (classified as comprehensive services)
Characteristics	Places great importance on family-style support and connections with the community	Provides integrated medical and nursing services

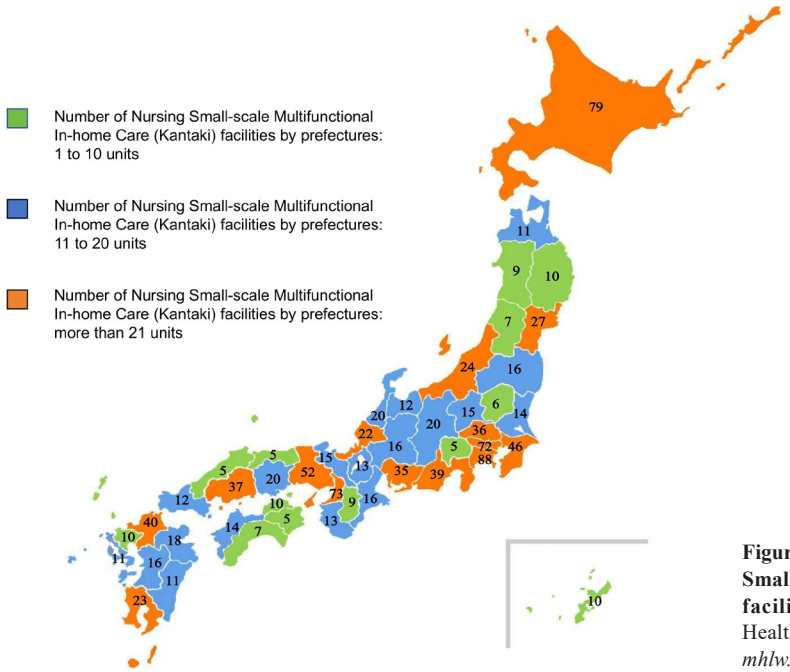

Figure 2. Distribution map of the number of Nursing Small-scale Multifunctional In-home Care (Kantaki) facilities by prefectures. (*Data Source*: Ministry of Health, Labour, and Welfare. *https://www.kaigokensaku.mhlw.go.jp*, as of July 2, 2025).

in densely populated urban areas rather than in regions with higher elderly population ratios. In sparsely populated areas, difficulties in recruiting staff and higher per-capita service delivery costs may act as significant barriers to implementation. Moreover, the survey also pointed out that many smaller municipalities still lack even a single "Kantaki" facility, reflecting a service coverage gap. Both "Kantaki" and "Shotaki" facilities face the issue of "implementation voids", as pointed out in a study by Kamiwada *et al.* (*26*). Such facilities have yet to be established in, many regions.

4. Problems with "Kantaki"

4.1. Limited awareness of "Kantaki"

A survey of community residents revealed a generally low level of awareness regarding "Kantaki". As many as 63.3% of respondents indicated that they had "never heard of it", highlighting a significant gap in public recognition of this service (*27*). However, among those who were aware of "Kantaki", approximately half expressed a willingness to use it, suggesting that there remains substantial latent demand for this type of care. Moving forward, exploring strategies to enhance public awareness will be essential — clarifying what "Kantaki" facilities are, what services they offer, and how they can serve as a viable option for those in need of medical and long-term care within the community.

4.2. Insufficient nursing staff

The stagnation in the promotion of "Kantaki" services is not solely due to limited awareness among users but

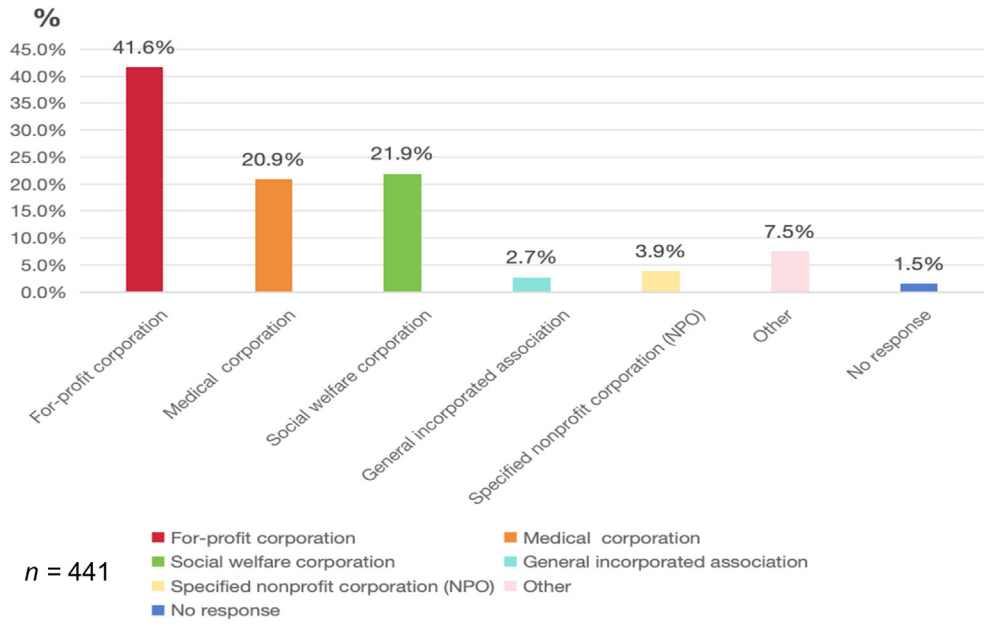

Figure 3. The operation of Nursing Small-scale Multifunctional In-home Care (Kantaki) facilities. (*Data Source*: *https://www.murc.jp/wp-content/uploads/2024/04/koukai_240425_13.pdf*).

also reflects limited understanding among healthcare professionals themselves, as well as a shortage of personnel with the flexibility and adaptability required for this type of care (*28*). In this new service model, how nurses should practically provide care remains unclear, making it a critical issue to address. Insufficient nursing skills have been associated with difficulties in handling complex tasks such as adjusting care plans or prioritizing needs, contributing to reduced self-efficacy among nurses (*29*). In reality, "Kantaki" nurses are required to provide a wide range of services, including assessing the necessity of medical consultations, responding to emergencies, and flexibly coordinating care arrangements. One study has indicated that nurses working in "Kantaki" facilities tend to experience low levels of self-efficacy (*30*). Improving nursing skills not only enhances professional autonomy but also helps increase nurses' self-efficacy (*31*). To promote the wider adoption of "Kantaki" services, nurses need to fully understand the scope of services they are expected to provide, their professional identity needs to be established, and their practical competencies need to be enhanced. Moreover, in order to improve job satisfaction and support the advancement of "Kantaki" services, tailored education and training programs should be developed based on the specific needs and capabilities of each facility. As a practical approach, online training programs to enhance nursing skills could be implemented, serving as a foundation for building a regionally based nursing support system.

4.3. Operational challenges

The operation of "Kantaki" services is primarily undertaken by five types of legal entities: for-profit corporations, medical corporations, social welfare corporations, general incorporated associations, and specified nonprofit corporations (NPOs), as shown in Figure 3. Among these, for-profit corporations account for the largest share and constitute the main providers of "Kantaki" services. This distribution reflects the active involvement of private-sector entities in the expansion of community-based care and underscores the need for robust quality management and mechanisms of regulatory oversight.

According to Katahira *et al*. (*32*), one of the primary challenges faced by "Kantaki" services lies in the demanding nature of their operational structure, and particularly the need to manage irregular and overnight shifts. These burdens often result in persistent difficulties in attracting and retaining adequate care personnel. Such staffing shortages not only increase the workload of frontline workers but also jeopardize the quality and continuity of care, thereby threatening the sustainability of facility operations. To ensure the stable management of "Kantaki" services, a robust staffing system needs to be established, especially with regard to overnight care, which can effectively reduce the burden on caregivers, improve job satisfaction, and foster healthier working conditions. As Hayama *et al*. (*33*) have also emphasized, the development of care organizations depends not only on innovative service models but also on the establishment of a solid institutional framework. The overnight care system in particular serves as a critical "lifeline" for operational stability and should be prioritized in both policy design and workforce development. Future efforts should therefore focus on enhancing institutional

support for night-shift care systems to ensure service continuity, safety, and professionalism.

4.4. Multi-role integration

"Kantaki" facilities rely heavily on multidisciplinary collaboration involving nurses, care workers, rehabilitation therapists, visiting physicians, and care managers to deliver integrated and person-centered care. However, due to differences in professional backgrounds, role recognition, and the lack of structured communication systems, collaboration among these professionals is often fragmented. For instance, discrepancies between nurses and care workers regarding the assessment of clients' health status or emergency responses can hinder care continuity and service integration (*32*).

Moreover, many "Kantaki" facilities operate on a small scale and often lack dedicated care coordinators, which further impedes the timely sharing of information and role clarity within the team. A study has emphasized that clear delineation of professional roles, regular multidisciplinary meetings, and shared documentation systems are essential for effective team-based care and for meeting the individualized needs of frail older adults (*34*). To enhance the quality and consistency of care, crucial tasks are to promote interprofessional education, establish collaborative training programs, and implement digital platforms for the seamless exchange of information. Systematic approaches to coordination will be critical in enhancing the ability of multidisciplinary teams to respond to the growing complexity of geriatric care needs in community settings.

5. Expectations and challenges for the future

"Kantaki" plays a central role in Japan's community-based integrated care system, offering coordinated services such as day care, short-term residential care, at-home nursing, daily living support, and basic medical care. As the country becomes home to a super-aged population with a high mortality, "Kantaki" will be increasingly essential to facilitate "aging in place" and provide end-of-life care. However, significant challenges remain in expanding and optimizing its implementation.

A persistent shortage of human resources remains a critical bottleneck in the development of "Kantaki". This challenge is particularly acute in small and mid-sized localities, where the recruitment of qualified nurses, physicians, and interdisciplinary care professionals is often difficult, compromising the sustainability of service provision. As a central pillar of the "Kantaki" system, the nursing profession contributes not only to the delivery of medical care but also plays a vital role in interdisciplinary collaboration, end-of-life support, and community building. Going forward, an essential task will be to strengthen the social foundation that maximizes the potential of "Kantaki" through the enhancement of nursing education, institutional reforms, and regional cooperation.

Despite its emphasis on multidisciplinary teamwork, "Kantaki" facilities frequently encounter operational challenges such as ineffective communication, unclear demarcation of roles, and insufficient mutual understanding among professionals, which limit the effectiveness of integrated service delivery. In response, the utilization of information and communication technologies (ICT) may offer solutions by enabling real-time visualization and efficiency-oriented management of care processes.

Moreover, significant regional disparities in facility distribution remain evident. While urban areas have made steady progress in establishing "Kantaki" services, development in sparsely populated regions has lagged due to higher operational costs and workforce shortages, resulting in persistent care "gaps". To address this, policy efforts should aim to implement incentive mechanisms that promote the equitable development of services across regions, particularly by supporting the establishment and sustainable operation of facilities in underserved areas.

Through the integrated implementation of these measures, "Kantaki" is expected to play an increasingly vital role in Japan's future elderly care system, helping to provide accessible, continuous, and person-centered community care.

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Review

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Human resources in long-term care for older adults in China: Challenges amid population aging

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SUMMARY: Against the backdrop of accelerating global population aging, China is undergoing significant demographic shifts. Its population aged 60 and above has reached 264 million, projected to account for 40% of the total population by the mid-21st century, becoming a "super-aging society" and triggering a surge in long-term care demand. On the demand side, the overall ADL disability rate among middle-aged and older adults is 23.8% (35.4% organic), rising to 30.5% among those aged 80 and above; 17.8% have IADL impairments, and 36.44% of households with older adults are empty-nest. Combined with population aging, rising disability rates, the growth of empty-nest families, and heavy disease burdens, care demand continues to grow annually. On the supply side, 13 million caregivers are needed for disabled/semi-disabled older adults, with only ~1 million practitioners; traditional models focusing solely on basic daily assistance fail to meet diverse needs like mental health support and rehabilitation. To this end, this study aims to synthesize evidence on the structural challenges faced by China's geriatric care workforce. By analyzing demographic data, care demand indicators, and geriatric care models, it identifies core issues and proposes evidence-based strategies, with the purpose of improving the quality of life of older adults and strengthening development of professional geriatric care talent.

Keywords: Population aging in China, geriatric care, shortage of nursing resources, older adults' care needs, older adults care models

1. Introduction

The World Health Organization (WHO) defines healthy aging as a process of developing and maintaining functional ability required for older adults to live a healthy life, with the goal of enabling them to live a dignified and quality life in their later years and reducing dependency (*1*). However, the intensification of global population aging, coupled with the increase in age-related diseases and rise in disability rates, poses significant challenges to global health and social care services (*2,3*). In 2020, the proportion of the population aged 65 and above reached 20% or more in 22 countries and regions; by 2050, the population aged 60 and above (2.132 billion) will be nearly twice the size of the adolescent population aged 15 to 24 (*4,5*).

Population aging and rising incidence of diseases among older adults have driven a surge in global demand for older adults care, with the imbalance between supply and demand emerging as a common issue. From the demand side, dependency on older adults care among older adults has increased significantly. Activities of Daily Living (ADL), which form the foundation for older adults to maintain daily independence, include basic behaviors such as eating, dressing, bathing, getting in and out of bed, and using the toilet; difficulty in performing these independently is considered disability, and the Instrumental Activities of Daily Living (IADL). It focuses on an individual's ability to independently complete complex daily tasks, and is a crucial reference for determining whether a person can live independently (*6*). Specific data show that the proportion of older adults with functional dependency in the UK is projected to increase by one-third between 2015 and 2035 (*7*); the

disability rate among older adults in the US has been rising (*8*), with ADL limitations intensifying across the 50-80 age group (*9*); one in five older adults in Japan is projected to have dementia in the future (*10*).

From the supply side, the prominent issue is a shortage of care resources. Studies indicate that the population aged 65 and over in the US will double by 2060 (*11*), leading to insufficient numbers of older adults care workers. Among the 4.5 million care providers, 86% are female with generally low educational attainment, resulting in a mismatch between supply and demand (*12*). Data from the Ministry of Health show that Japan's demand for long-term care workers will rise from 2.11 million in 2019 to 2.8 million in 2040, and with children of the baby boomer generation retiring around 2040, pressure on the supply of care workers will persist (*13*).

Compared with global trends, population aging in China, characterized by a larger scale and faster speed, endows the contradiction between supply and demand in care provision with greater particularity and urgency. As a large developing country accounting for nearly one-fifth of the world's population, addressing population aging constitutes a severe challenge, and whether older adults receive adequate care services is also a cause for concern. In 2024, the population aged 65 and above in China reached 220 million, accounting for 15.6% of the total population, and it is projected to rise to 26% by 2050 (*14*). Additionally, the proportion of the population aged 65 and above increased from 7% to 14% in only 21 years, compared with 115 years in France (*15*).

Superimposed with national conditions such as family downsizing caused by the family planning policy, "aging before becoming wealthy", and "aging before being prepared", the contradiction between supply and demand of care resources has been exacerbated, while health status of the older adult population has further intensified this contradiction. Data show that the overall ADL disability rate among middle-aged and older adults in China reaches 23.8% (35.4% of which are organic) (*16*). There are 33 million people aged 60 and above with difficulties in daily living, among whom nearly one-third need to depend on others for care (*17*).

Caregivers are the core link connecting the demand for care for older adults and health outcomes, and their capabilities directly affect the healthy life expectancy of older adults. However, the workforce of geriatric caregivers in China is characterized by insufficient quantity, weak professional qualifications, imbalanced personnel structure, and poor stability. Based on the needs of disabled and semi-disabled older adults, 13 million caregivers are required, but there are only about 1 million actual practitioners, among whom 300,000 have received professional training and 100,000 hold professional qualifications. The workforce is predominantly female (over 80%) and aged 40–60, with low educational attainment (two-thirds having junior high school education or below, and only 7.8% having junior college education or above), and the rate of certified employment is less than 32% (*18,19*). These structural deficiencies lead to poor stability. Surveys show that caregivers work over 10 hours per day on average, with a monthly income of less than 5,000 Chinese yuan; 61.1% are dissatisfied with their work, 51.8% have an intention to resign, and average turnover rate is 23.3%, which exacerbates manpower shortages and hinders development of long-term care services (*20-22*).

The shortage of geriatric caregivers has imposed pressure on the social older adults care service system. Against this backdrop, this study, set against the context of population aging in China, focuses on the predicament of "insufficient quantity, low quality, and poor stability" in the geriatric care workforce. It synthesizes current situations and issues (including care models, actual demand, contradictions between health heterogeneity and care provision, and structural shortcomings of the system), aiming to provide references for workforce development and system improvement.

2. The Current Situation of Aging in China

2.1. Changes in the total population of older adults in China

From 2013 to the end of 2023, the total number of older adults aged 60 and above in China reached approximately 290 million, accounting for 21.1% of the total population (Figure 1). Over these 10 years, the number increased by 100 million, with an average annual growth of 6.7 million. The proportion of older adults in the total population rose from 10.45% to 18.75% (*23*). Nationwide, the number of older adults aged 65 and above stood at 216.76 million, making up 15.4% of the total population (*24*).

A comparison of data from the Fifth and Sixth National Population Censuses shows that the proportion of older adults aged 60 and above in the total population increased by 2.86 percentage points (*25*). Between the Sixth and Seventh National Population Censuses, this proportion rose by 5.44 percentage points (*26*), indicating that China's population aging process has accelerated since 2010. This acceleration is an inevitable outcome of the family planning policy implemented in 1982: individuals who were of childbearing age (20–30 years old) at that time have now reached 50–60 years of age. Consequently, the proportion of older adults has continued to rise since 2020, posing new demands and challenges for economic development and urban management. Notably, demand for care among older adults has become increasingly prominent.

2.2. Changes in age structure of the older adult population in China

With increasing life expectancy, the numbers of

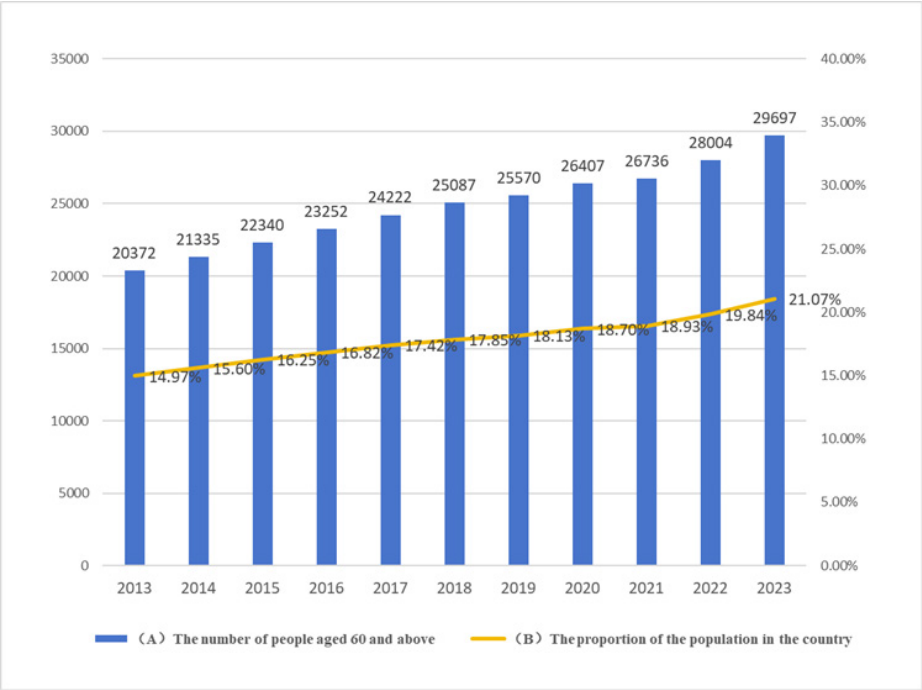

Figure 1. The total number of Chinese older adults aged 60 and above from 2013 to 2023. In the figure, these two sets of data represent: (A) number of people aged 60 and above; (B) proportion of older adults population in total national population. *Data source*: 2023 Annual Report on the Development of older adults Affairs in China (*https://www.gov.cn/lianbo/bumen/202410/content_6979487.htm*).

advanced older adults, long-lived older adults, and centenarians have continued to rise. From 2000 to 2010, the number of older adults aged 70 and above increased from 53 million to 78 million. From 2010 to 2020, this figure further grew from 78 million to 117 million, with its proportion in the total population rising from 4.29% to 5.83% and then surging to 8.29%. From 2000 to 2020, the number of advanced older adults aged 80 and above increased from 8.96 million to 24.97 million, and their proportion in the total population rose from 0.71% to 1.78%. Over the same period, the proportion of long-lived older adults aged 90 and above also increased from 0.07% to 0.33%, while the number of centenarians grew from 18,000 to 119,000. These trends indicate that the growth of China's advanced older adults population is accelerating. Advanced aging is often accompanied by issues such as empty-nest living, disability, cognitive impairment, poverty, and multimorbidity, rendering the challenge of daily living care particularly prominent (*27*).

2.3. Marital status of older adults in China

According to data from the Fifth, Sixth, and Seventh National Population Censuses, the proportion of unmarried individuals aged 15 and above in China decreased from 20.2% to 19.2% between 2000 and 2020. Among this group, the proportion of unmarried older adults aged 60–64 dropped from 2.2% to 1.7%, while the proportion of unmarried older adults aged 65 and above rose slightly from 1.4% to 1.6%. The proportion of divorced individuals increased from 0.9% to 2.4% over the same period. Specifically, the proportions of divorced older adults aged 60–64 and those aged 65 and above rose by 1.5 percentage points and 0.4 percentage points, respectively. The proportion of widowed individuals increased marginally from 5.6% to 5.7%. Although the proportion of widowed older adults aged 60 and above declined to some extent, it is noteworthy that the proportion of widowed older adult women remains high, reaching 37.8% by 2020 (Table 1) (*25,26*).

2.4. Living arrangements of older adults in China

Currently, older adults living in empty-nest families account for 36.44% of the total older adult population, with the primary living arrangement being that older adult couples (aged 65 and above) reside independently (Table 2) (*28*). In terms of living arrangements for older adults, home-based care holds an absolutely dominant position, accounting for 98.6%, whereas institutional care accounts for only 1.4%. The combined proportion of couple households and single-person households stands at 52.3%, representing a 12.7% increase compared with 2010 (*29*). Additionally, the proportion of empty-nest older adults in rural areas is higher than that in urban areas. Nationwide, in households with only one older adult, the proportion of single older adults living alone exceeds one-third. Both at the national level and across urban and rural areas, the proportion of single older adults living in one-person households (single-person households) is gradually increasing (*30*).

Table 1. Marital status proportions of the population aged 60 and over in China (2000–2020) (%)*

Category	Unmarried			Divorced			Widowed		
	2000	2010	2020	2000	2010	2020	2000	2010	2020
Total	20.2	21.6	19.2	0.9	1.4	2.4	5.6	5.7	5.7
60–64	2.2	1.9	1.7	0.8	1.0	2.3	14.8	11.5	8.3
≥ 65	1.4	1.7	1.6	0.6	0.7	1.0	37.7	34.5	27.1

*Population aged 60 and above in China (2000–2020). Data Source: *Ref.* (*2,7,8*).

Table 2. Living style and changes of the older adults (%)*

Category	2020		2010		2000	
	Live alone	Couples living alone	Live alone	Couples living alone	Live alone	Couples living alone
Country	36.44	55.96	24.28	47.92	15.79	41.99
City	34.86	56.80	26.25	52.19	18.52	46.76
Town	36.33	55.33	25.45	50.49	18.26	47.40
Village	37.43	55.64	23.15	44.83	14.56	39.30

*Population aged 65 years and above (2000-2020). Data Source: *Ref.* (*12*).

2.5. Health status indicators and trends among older adults

With the intensification of population aging, the proportions of illness, medical consultation, and hospitalization have also increased significantly. According to the results of the 1st to 6th Health Service Surveys, the two-week prevalence rate, two-week consultation rate, and hospitalization rate among China's older adult population have all shown a rapid upward trend, rising from 25% to 58.4%, 28.0% to 42.6%, and 6.1% to 27.2% respectively. As age increases, the proportions of hearing impairment, visual impairment, and dementia also gradually rise, standing at 57.1%, 48.7%, and 7.8% respectively, with a significant increase after the age of 80 (Figure 2) (*31,32*).

2.6. Care models for older adults in China and current status

China's older adults care model has evolved with social development: from traditionally family-centered, to state-subsidized and collective-supported care, to today's socialized, diversified model integrating family, community, and institutional care. Compared with mature systems in other countries, China's remains exploratory (*33*). Though a diversified framework is initially formed, supply-side fragmentation is prominent, characterized by "rapid growth of institutional care and relative lag in home and community-based services (HCBS)" (*34*).

Family care, once primary for older adults, has weakened with shrinking families (1.6 adult children per urban older adult on average). Most prefer home care, but support is insufficient (*35*). A 2024 survey revealed that only 15.3% of disabled older adults aged 65 and above have access to family care, failing to address large-scale care demands (*36*). Community care (home visits, day care centers) grew rapidly, with beds rising from 198,000 to 3.478 million (2012–2018). However, rural areas lag due to scarce resources and scattered populations, with lower coverage of professionals and facilities than cities, failing to fill family care gaps (*34*).

Institutional care, as a professional carrier for addressing complex care needs (particularly for disabled older adults), has become increasingly prominent. By 2023, there were over 400,000 older adult care institutions nationwide, housing approximately 8.5 million older adults, 70% of whom were disabled or semi-disabled. These institutions are major employers of professional geriatric caregivers, absorbing over 60% of the national geriatric care workforce, and their development directly impacts stability and service quality of the care team (*37*).

A survey on Chinese older adults showed that 86.37% preferred living at home, but their inclination toward institutional care grew with increased disability (*38*). However, institutional care faces structural challenges: while beds rose from 2.345 million in 2008 to 7.271 million in 2018 (from 21.4 to 43.6 beds per 1,000 older adults aged 65 and above), bed vacancy rates remained high (reaching 55.1% in 2014). Shortages of professional nurses and insufficient service standardization further constrained its role in attracting and stabilizing the care workforce (*34*).

3. Contradiction between care needs of older adults and insufficient supply in China

3.1. Current situation of actual care needs of older adults

Older adults in China are characterized by advanced age, chronic diseases, disability, and empty-nest status. Rising disease prevalence has directly driven rapid growth in

demand for older adults care, long-term medical services, and nursing care (*39,40*). Relevant studies indicate that the trend of functional dependence among older adults has hindered health and social care planning and resource allocation; national sampling surveys on persons with disabilities also show an upward trend in disability prevalence among individuals aged 60 to 74 (*41*).

In terms of current health and disability status, the Seventh National Population Census shows that 12.7% of older adults aged 60 and above are in poor health, and 2.3% are unable to manage daily life independently. Those living alone or in older adults care institutions have poorer health than those living with family, which may be related to care dependence caused by poor health (Table 3) (*23*). The Sixth Health Services Survey further notes that 9.3% of the older adult population are fully self-reliant, with proportions of mild, moderate, and severe disability at 3.7%, 1.1%, and 1.8% respectively. The disability rate is higher in rural areas than in urban areas, and among older adults aged 80 and above, the proportions of mild, moderate, and severe disability reach 10.3%, 3.5%, and 6.2% (Figure 3) (*31,42*).

In terms of activities of daily living, the ADL disability rate among older adults aged 60 and above was 7.8% in 2020 (reaching 30.5% among those aged 80 and above), with an IADL impairment rate of 17.8% (21.3% in rural areas, higher than 18.1% in urban areas). Additionally, the prevalence of dementia among older adults aged 65 and above was 5.14%, with higher risks observed in rural populations and illiterate groups (*36*). Currently, 17.8% of older adults require care, and the unmet rate of ADL needs reached 54.3% in 2020 (*43*). The Sixth National Health Services Survey shows that the proportion of older adults needing assistance in six daily activities (including bathing and dressing) ranged from 1.8% to 5.7%. Among those receiving older adults care services, preventive healthcare accounted for the highest proportion (30.4%), while 56.8% received no

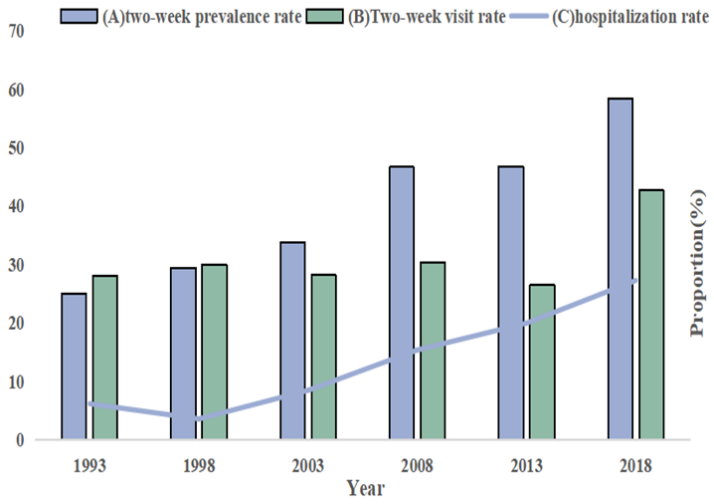

Figure 2. Morbidity and medical treatment among older adults, 1993–2018. In the figure, the three kinds of data represent respectively: (A) two-week prevalence rate; (B) Two-week visit rate; (C) hospitalization rate. The calculation method for the two-week prevalence rate is the number of patients among the older adults population within two weeks divided by the total number of the surveyed older adults population. Two-week visit rate is equal to number of visits by the older adults population within two weeks in the survey/the total number of the older adults population surveyed. Hospitalization rate is equal to the number of hospitalizations of the older adults population within one year in the survey/ the total number of the older adults population surveyed. *Data Source*: The Sixth National Health Service Statistical Survey Report of China (2018) (*https://thinker.cnki.net/bookstore/book/bookdetail?bookcode=9787117312813000&type=book*).

Table 3. Health status of the older adults aged 60 and above (%)

Category	Health	Basically healthy	Unhealthy but able to take care of oneself in daily life	Unhealthy and unable to take care of oneself in daily life
Live with spouse and children	64.3	27.2	6.9	1.6
Live with spouse only	57.6	32.3	8.5	1.6
Live with children only	44.0	36.4	15.1	4.6
Live alone with a nanny	28.8	31.2	18.6	21.4
Live alone without a nanny	43.2	39.2	16.2	1.3
Live in a nursing home	13.0	30.7	29.6	26.7
Others*	53.7	31.8	10.8	3.7
Total	54.6	32.6	10.4	2.3

*Others: Living with relatives / Living in a collective residence/temporary living. Data Source: *Ref*. (*2*).

services. Moreover, the more severe the disability, the higher the proportion of receiving rehabilitation care and daily living assistance (Table 4) (*26*).

In terms of mental health, the decline in physical function can exacerbate psychological burden, and late-life depression can also increase the prevalence of physical illnesses and further reduce quality of life (*44*). Currently, the overall prevalence of depression among older adults is 22.7%, with rural women (47%) having a significantly higher rate than urban men (22%), primarily associated with social isolation (*36*). A survey in Northeast China shows that 81.66% of older adults have needs for psychological support, and the detection rate of depression among empty-nest older adults (34.2%) is significantly higher than that among non-empty-nest older adults (18.5%) (*45*). A multi-center study across 17 provinces indicates that among the primary care needs of older adults, mental health (76%), hospice care (73%), and older adults care environment (71%) rank as the top three (*46*).

Social participation, as a key measure for actively responding to population aging, is crucial for alleviating psychological issues and enhancing quality of life. Studies have confirmed that it can reduce the risk of depression, slow down cognitive decline, and protect the mental health of older adults (*47,48*). However, there are significant disparities in its current status. Among 7,901 respondents, the participation rate in economic activities is the highest (55.14%), while participation rates in socializing (31.97%) and exercising (7.4%) are relatively low, constrained by factors such as environment and physical conditions (*49*). Currently, the demand of older adults for Older Adults care services has shifted from mere material security to equal emphasis on material and spiritual needs, with particular attention to rehabilitation care and cultural and recreational activities. They are in urgent need of reliable care, emotional comfort, and diversified leisure activities (*50*).

3.2. Impact of changes in family structure on caregiving functions

Currently, home-based care for older adults remains the primary form of older adults care in China (*51*). With the decline in family size and increased life expectancy, the likelihood of both children and parents entering older age

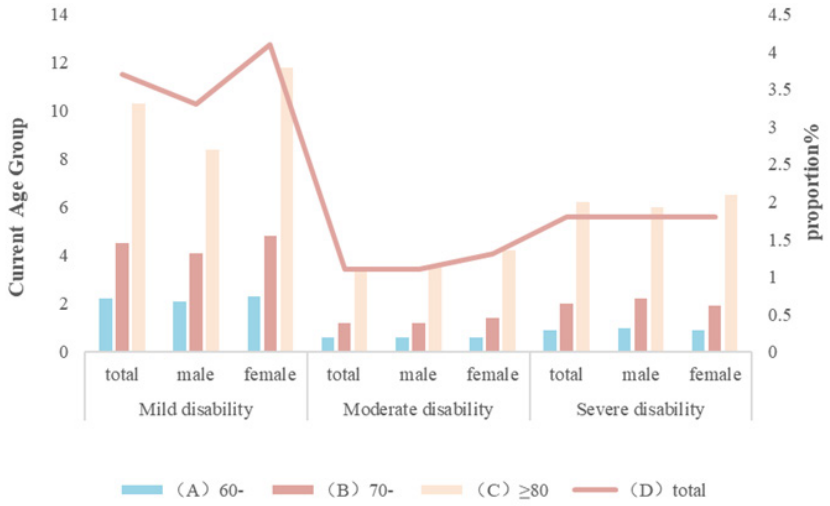

Figure 3. Health Status of older adults at Different Ages (%). In the figure, the three kinds of data represent respectively: (A) The varying degrees of disability among people of different genders aged 60 and above; (B)The varying degrees of disability among people of different genders aged 70 and above; (C) The varying degrees of disability among people of different genders aged 80 and above. We calculated the number of older adults aged 60 and above, 70 and above, and 80 and above respectively, and then calculated the disabled older adults of different grades among men and women respectively corresponding to the population numbers of different grades. *Data Source*: The Sixth National Health Service Statistical Survey Report of China (2018) (*https://thinker.cnki.net/bookstore/book/bookdetail?bookcode=9787117312813000&type=book*).

Table 4. Current situation of care services for older adults with different levels of disability (%)

Degree of disability	Preventive health care	Medical assistance	Rehabilitation nursing	Spiritual solace	Daily living care	Cultural and sports activities	Education for older adults	None
Fully self-care	36.8	16.9	2.2	2.2	2.8	5.8	10.5	67.9
Mild disability	32.9	16.7	3.0	2.4	5.3	2.4	7.8	69.9
Moderate disability	28.3	17.4	4.9	3.0	5.2	0.8	7.8	75.6
Severe disability	30.1	16.5	6.4	2.4	7.2	0.5	6.5	74.8
Total	30.4	14.1	1.9	1.8	2.5	4.6	8.6	56.8

Data Source: *Ref*. (*26*).

simultaneously has risen. The main family caregivers are shifting from young and middle-aged adults to middle-aged and older individuals (*52*), with scenarios such as younger older adults caring for older adults and middle-aged people providing care for multiple older adults becoming prevalent realities and dilemmas (*53*,*54*).

China's home-based care model for older adults faces structural difficulties. Amid shrinking families and longer life expectancy (*55*), "younger older adults caring for older adults" and "middle-aged people caring for multiple older adults" are common, with main family caregivers shifting from young adults to middle-aged and older individuals. Among mildly disabled older adults, 75.26% and moderately disabled ones 66.42% choose home/community care; rural disabled older adults rely on family care (92.7%) over institutions (1.4%) (*56,57*). For disabled older adults aged 80 and above, 45.3% receive spousal care, while adult child caregivers (average 52.7) face heavy physical and mental stress (*58,59*). Under "4-2-1" family structures, middle-aged people bear dual responsibilities of supporting 2–3 older adults and raising children, reducing care quality and increasing adult children's economic burdens (*60,61*). Only 32% of 1.27 million care workers are certified, with a 38% gap in long-term care demand for disabled older adults; while insufficient care supply and talent shortages restrict institutional care (*62*). Additionally, rural adult children's migration has significantly reduced rural family care availability (*45*).

In addition, according to the Report on Research of Urban Home-based Care Services for Older Adults released by the National Committee on Aging, the proportion of empty-nest families (including older adults living alone) among the urban older adult population has reached 49.7%, and 56.1% in large and medium-sized cities, with older adults living alone accounting for 12.1% (*63*). Most of these older adults are unable to manage daily life independently and require substantial care, attention, and medical resources (*64*).

The growing number of older adults who are advanced in age, disabled, or living in empty-nest situations has placed significant pressure on China's social care service system for older adults, coupled with a severe shortage of care workers for older adults. Therefore, there is an urgent need for professional care teams and socialized care services to address these issues.

3.3. High costs and access barriers in long-term care

China's National Assessment Report on Aging and Health Status notes that approximately 33% of the country's disease burden stems from older adults' health issues (*65*). With rising per capita burden of chronic diseases, older adults in China face higher disease burdens than those in other low-income countries. The number of semi-disabled older adults is over twice that of disabled ones. Projections suggest that by 2050, semi-disabled older adults aged 80 and above will reach around 100 million (annual growth ~3%), and disabled ones 20.72 million (annual growth 3.7%). These groups require more long-term care than ordinary older adults, imposing heavier disease and care burdens (*36,66*).

High and persistent long-term care costs for disabled older adults are a key barrier to adequate care. Scholars studying direct long-term care costs note that nursing costs for disabled older adults are over twice those of age-matched fully functional peers. Semi-institutional care (2.65227 trillion Chinese yuan) costs over twice home care (1.23494 trillion Chinese yuan), and full institutional care (3.99877 trillion Chinese yuan) over three times (*67*). A study on such costs found home care for disabled older adults accounts for 19.4% of household weekly income, rising to 90.1% for community-institutional care and 134.4% for institutional care — costs often unaffordable for families (*57*). China's urban and rural resident medical insurance covers only hospitalization and special nursing expenses, excluding long-term care costs, exacerbating hardships for disabled older adults. Additionally, home care limitations (inability to perform certain procedures) force repeated hospitalizations, causing admission difficulties and higher medical costs.

Per capita medical expenses for those aged 65 and above are 2–8 times higher than for people under 65, with over 80% of lifetime medical costs incurred by individuals aged 60 and above (*68*). As the number of disabled older adults rises, nursing, support, and medical costs will increase, imposing heavy burdens on families and society. A *Lancet* report notes that by 2030, 14.02 million older adults in China will need long-term care, creating a substantial funding gap (*45*). Seventh National Population Census data show older adults relying on labor income or pensions have better health than those dependent on unemployment insurance, family support, or minimum living allowances — particularly in rural areas. This highlights the need to strengthen safeguards for older adults with unstable livelihoods and low living standards.

3.4. Development of care institutions for older adults and gaps in service supply

As the number of disabled older adults in China grows, families and society face greater long-term care responsibilities, with disabled older adults showing growing willingness to reside in care institutions long-term. Data shows that by late 2020, there were 5,857 fully licensed medical-nursing integrated institutions, up 59.4% from late 2017. Additionally, 72,000 contracted partnerships existed between medical institutions and older adults care services — 6.1 times the number in late 2017.

However, despite the growing number of medical-nursing integrated institutions, care institutions for older adults still have persistent deficiencies in medical service

capabilities (*69*). Most lack adequate public activity facilities, particularly medical service facilities — nearly 50% of Xi'an's care institutions for older adults lack such medical facilities (*70,71*). Basic configuration of basic medical facilities, fundamental to providing medical and nursing services, directly affects these institutions' service supply and functional performance. Care institutions for older adults face issues including low occupancy rates, heavy government dependence, and weak internal motivation. Additionally, public institutions receive greater policy and financial support, while private ones suffer from caregiver shortages and low caregiver quality (*72*). Studies show nursing bed occupancy dropped significantly from ~80% in 2008 to ~55% in 2014. For private institutions, low occupancy stems from high costs, lack of insurance coverage, inadequate services/facilities, poor care quality, and inconvenient locations (*73*).

China's long-term care for older adults has long seen a severe supply-demand imbalance, worsened by shifting family structures. With mounting challenges, traditional home-based care has weakened amid caregiver aging and scattered care resources. In this context, institutional care — with its professional service advantages — is shifting from a supplement to a key pillar of the older adults care system (*46*). Future demand for long-term care will grow, with rising shares of disabled, advanced-age, and empty-nest older adults. However, institutional care development is constrained by shortages of professional nursing talent. This requires expanding institutional care supply, improving staff professionalism, and refining talent cultivation mechanisms to meet older adults' diverse needs for quality medical and health services (*50*). Shifting from family dependence to socialized, professional services is not only inevitable for addressing aging but also critical for sustainable development of the older adults care system.

4. Current situation of older adults caregivers in China

In 2019, the national Notice on Strengthening Geriatric Nursing Services proposed the need to comprehensively integrate geriatric nursing resources, increase the number of medical institutions providing geriatric nursing services, and encourage grassroots medical and health institutions with the necessary conditions to set up and add beds for geriatric nursing services as required. Tertiary hospitals are encouraged to mainly provide specialized nursing services for older adults patients and undertake tasks such as technical support for geriatric nursing and talent training (*74*). Against the backdrop of the global trend of population aging, the aging process in China is accelerating continuously, and the scale of the older adults population who are disabled or living alone is growing increasingly large. This profound change in the population structure has made the older adults care model that deeply integrates medical care and nursing a key measure to cope with an aging society, and it has also put forward higher requirements for the professionalization level of geriatric nursing (*75,76*). High-quality geriatric nursing services cannot be achieved without support of a professional team. However, currently, there are obvious shortcomings in construction of China's geriatric nursing team. Especially, the group of nursing assistants is characterized by a low level of education and a lack of professional knowledge, which clearly fails to meet the needs of China's aging society (*75,77*). Some studies have pointed out that in 2015, there were 2.147 million service recipients in older adults care service institutions nationwide. According to the staffing standards of 3:1 for disabled older adults people and professional nursing staff and 10:1 for self-care older adults people and professional nursing staff, the number of required service personnel was approximately 350,000, but the actual number of service personnel was only 195,600 (*78*).

From this, it can be seen that the shortage of geriatric nursing talents in China has become the core bottleneck restricting construction of the older adults care service system. If the bottleneck in talent cultivation and reserve cannot be broken through as soon as possible, the older adults care model that deeply integrates medical care and nursing will be difficult to promote due to the weakness of professional strength. Care needs of the large number of disabled and older adults people living alone will also face a more severe supply gap, posing a significant challenge to the sustainable development of an aging society (*34*). The diversified needs of the older adults put forward higher requirements for cultural knowledge, and demand higher professional skills, service levels, qualifications, and professional qualities of medical staff. Therefore, it is necessary to encourage cooperation with institutions of higher learning, strengthen talent reserve, and cultivate professional qualities of geriatric care personnel (*79*).

4.1. Structure and distribution of geriatric departments

The data on the settings of geriatric-related departments were analyzed through the "Electronic Registration Information System for Medical Institutions" (*80*). A total of 444,000 various departments were set up in public hospitals in 31 provinces (autonomous regions and municipalities directly under the Central Government) across the country, among which 3,394 were geriatric-related departments, accounting for 0.76% of the total number of departments established in the 31 provinces (autonomous regions and municipalities directly under the Central Government). Proportions of geriatric-related departments in general hospitals, traditional Chinese medicine hospitals, specialized hospitals, sanatoriums and nursing homes were 0.59%, 0.92%, 1.50%, 1.12% and 8.78% respectively. Overall, specialized hospitals, sanatoriums and nursing homes had relatively

high proportions of geriatric departments. In terms of provinces, Qinghai Province, Shanghai City and Chongqing City had proportions of geriatric departments reaching 2.01%, 1.94% and 1.31% of the total number of departments respectively. In these three provinces, the proportion of people aged 60 and above in Shanghai City and Chongqing City exceeded 20% (Table 5).

4.2. Current situation of caregivers in older adults institutions

As the core force of professional care, nursing staff in nursing institutions for older adults directly affect the service quality of institutions and play an important role in China's older adults care system. This profession is jointly approved and established by the Ministry of Human Resources and Social Security and Ministry of Civil Affairs, with a very low entry threshold. They mainly engage in daily living care for older adults, but currently face multiple issues such as low job satisfaction, high turnover rate, low social recognition, and mental health problems (*81,82*).

Studies have found that the quality of care for older adults is closely linked to nursing staff's job satisfaction (*83*). Nursing staff with higher job satisfaction tend to demonstrate better work performance, which positively influences their work commitment and intent to remain in the profession (*84*). However, job satisfaction among nursing staff caring for older adults in China is generally low, with key contributing factors including: emotional exhaustion, lack of personal accomplishment, and inadequate social security (*e.g.*, only 12% participate in "three social insurances" or "five social insurances", with insufficient coverage for unemployment and work-related injuries) (*85*); and insufficient protection of labor rights – a large-scale random sampling in Beijing in 2019 revealed that only 1.6% of nursing staff had signed labor contracts (*86*); and a severe imbalance between remuneration and work intensity — a survey in Zhejiang Province showed that 76.8% of nursing staff earned less than 2,500 Chinese yuan per month and worked over 10 hours daily (*87,88*).

This low satisfaction directly leads to a surge in staff turnover. Prolonged working hours, low salaries, heavy workloads, and emotional burdens contribute to high turnover rates among employees (*89*). Surveys show that nursing staff in older adults care institutions generally feel dissatisfied due to low pay — 96.26% earn 1,000–3,000 Chinese yuan per month — and a mismatch between salary and work intensity (*90*). Additionally, some institutions report an average turnover rate exceeding 30%, with the highest reaching 35.71% (*91*). Nursing staff in Chengdu scored (72.44 ± 18.22) on job burnout and (14.87 ± 3.77) on turnover intention (*92*), indicating significant overall turnover propensity and burnout, which further exacerbates labor shortages.

Prolonged high pressure also severely impairs the mental health of nursing staff. Issues such as heavy workloads, low social status, and weak family support lead to the accumulation of significant negative emotions (*93*). Scholars have found that 48.9% of nursing staff in older adults care institutions report having experienced discrimination; such subjective biases exacerbate emotional burdens and damage self-confidence and self-esteem (*94*). Additionally, studies indicate that the detection rate of job burnout among nursing staff reaches 51.43% (*93*), with 68% experiencing moderate to high emotional exhaustion, accompanied by depressive tendencies. This not only reduces work enthusiasm and personal accomplishment but also significantly increases psychological burden (*95*). In summary, the interlinked issues faced by nursing staff highlight the inadequacy of support for service providers in the current older adults care system. Urgent measures to safeguard their rights and interests are needed to promote the sustainable development of the older adults care workforce.

4.3. Main problems existing in the older adults care team from the perspective of policies and systems

Statistical standards and scope remain unclear. In the current system for care professionals for older adults, at the institutional statistical level, specialized hospitals for geriatric medicine are ambiguously categorized with other specialized hospitals; though general hospitals at or above Level 2 are required to establish geriatric medicine departments, these are not separately classified in statistics, resulting in unclear baseline figures for nursing personnel at the institutional level. At the personnel statistical level, only classification standards for registered geriatric medicine registered doctors are defined, while care staff in older adults care institutions, hospital-based older adults care workers, and primary care workers are not included in statistics. This directly leads to a lack of accurate data support for "the gap between the number of professionals and actual demand", increasing the difficulty of judging supply-demand imbalance (*63*).

In terms of career development, policy support for nursing staff is inadequate. China's 2002 National Occupational Standards for Older Adults Care Workers mandates middle school education and 180 hours of training for such workers, yet actual compliance rates are extremely low (optimistically estimated at less than 1/3) (*96*). Currently, there are no unified professional grading standards or promotion pathways for Older Adults care workers, with most reporting unclear career advancement directions. Studies show only 53.55% of nursing staff hold professional qualifications; among the 83.6% who have received training, merely 38.9% are satisfied with its quality. Additionally, professional training policies are fragmented, suffering from "three deficits": lack of opportunities (40.8%), poor suitability (35.7% cite unreasonable training timing/locations), and insufficient

Table 5. The setup situation of older adults-related departments in different hospitals of different provinces*

Province	General Hospital	TCM Hospital	Specialized Hospital	Ethnic Medicine Hospital	TCM-WM Hospital	Sanatorium/Nursing Home Station	Total of Geriatrics Departments	Total of Established Departments	Proportion of Older Adults-related Departments	Population Aged 60 and Above
Anhui	72	24	13			10	119	12,872	0.92	18.8
Beijing	36	7	16	1	6		66	9,315	0.71	19.6
Fujian	27	15	6				48	8,800	0.55	16.0
Gansu	41	36	12		1		90	9,534	0.94	17.0
Guangdong	108	35	50		9		202	30,296	0.67	12.4
Guangxi	37	13	24	1	8	6	89	17,215	0.52	16.7
Guizhou	19	15	3				37	9,433	0.39	15.4
Hainan	13	9				1	23	2,704	0.85	14.6
Hebei	148	58	29		4	4	243	31,078	0.78	19.9
Henan	103	78	21		1		203	30,191	0.67	18.1
Heilongjiang	66	17	1		6		90	15,100	0.60	23.2
Hubei	62	27	7		2	1	99	15,896	0.62	20.4
Hunan	66	53	83				202	22,860	0.88	19.9
Jilin	50	22	12		1		85	11,015	0.77	23.1
Jiangsu	60	33	36		5	9	143	15,673	0.91	21.8
Jiangxi	51	17	17			14	99	12,163	0.81	16.9
Liaoning	44	8	4	2			58	17,412	0.33	25.7
Inner Mongolia	36	11	7	10	7		71	10,324	0.69	19.8
Ningxia	9	1	1	1			12	2,799	0.43	13.5
Qinghai	8	59	2				69	3,435	2.01	12.1
Shandong	126	74	42		6	12	260	31,168	0.83	20.9
Shanxi	74	33	25		5	1	138	16,846	0.82	18.9
Shaanxi	60	22	12				94	14,669	0.64	19.2
Shanghai	69	14	59		3	8	153	7889	1.94	23.4
Sichuan	102	75	74	2	3		256	28,163	0.91	21.7
Tianjin	10	2	1		1		14	3,606	0.39	21.7
Tibet							0	1,571	0.00	8.5
Xinjiang	66	8	11	1	1		87	11,560	0.75	11.3
Yunnan	28	70	22				120	13,057	0.92	14.9
Zhejiang	63	18	40		11		132	20,751	0.64	18.7
Chongqing	49	26	16		1		92	7,037	1.31	21.9
Total	1,703	880	646	18	81	66	3,394	444,432	0.76	18.7

*Data on the department setup of public hospitals in the Electronic Registration Information System for Medical Institutions. Data Source: *Ref.* (*56*).

practicality (only 53.4% find content helpful for work). This hinders skill improvement, exacerbating issues of inadequate expertise and low professional value (*77*).

In terms of social status, there is a lack of institutional recognition and inadequate protection for nursing staff's social status. Professional positioning is ambiguous: policies do not classify "older adults care workers" as "professional and technical personnel" but rather as "social service personnel", creating an institutional gap in professional identity compared to medical staff. Additionally, care workers face "social stigmatization". Despite policy advocacy to elevate status of older adults care practitioners, the absence of supporting professional honor systems and public awareness mechanisms fails to effectively alter social prejudices, directly impacting social recognition (*97*). Policies are insufficient in enhancing care workers' social status, lacking unified career promotion systems and guidance for social recognition.

Outdated management and collaboration mechanisms hinder workforce effectiveness. Medical institutions, older adults care facilities, and family caregivers lack regular communication, impeding the circulation of professional knowledge. Personnel management has flaws: no unified national entry thresholds, fragmented training systems (only 58% of institutions offer systematic pre-service training), and outdated training content, and misaligning caregivers' skills with needs. For instance, merely 12% of training covers chronic disease management and 5% includes smart care equipment operation (*97,98*); and training suffers from insufficient opportunities (40.8%) and poor suitability (35.7%) (*99*). The absence of incentive mechanisms (70% of institutions not implementing "skill-based graded compensation") leads to a workforce plagued by "difficulties in recruitment, retention, and skill improvement", becoming an institutional barrier to better service quality (*100*).

5. Conclusion

With the intensification of population aging and changes in family structures, demand for Older Adults care services continues to evolve, making strengthening the older adults care workforce an urgent priority. To address issues and challenges in training older adults care workers, practical recommendations are proposed to optimize the care workforce system and improve service quality, including:

i) Enhancing top-level design. Develop a unified national plan based on national conditions, the demographic structure of older adults, and social needs; improve financial input mechanisms and incorporate them into local assessments. Unify professional standards for occupations such as care workers and rehabilitation therapists, and standardize qualification certification. Eliminate occupational prejudices through media publicity, include outstanding practitioners in national honor selections, clarify norms for the entire process of employment and promotion, and smooth career development pathways.

ii) Optimizing incentive mechanisms. Establish a unified national qualification certification system with "certification-based employment", linking assessments to salaries and promotions. Build a salary system matching labor input (senior care workers' pay not lower than 1.2 times the local average social wage), improve social security and allowances, and offer housing and children's school enrollment benefits to long-term practitioners. Set up special funds to recognize outstanding staff, with their families enjoying fee discounts for institutional care, addressing issues of "difficult recruitment and retention".

iii) Upgrading the training system. The Ministry of Human Resources and Social Security and the National Health Commission should jointly formulate training syllabuses, mandating coverage of comprehensive skills such as chronic disease management and smart device operation. Promote the "theory + practical training + rotation" model, set up training bases in communities, and require care workers to participate in at least 80 hours of continuing education every two years, with delayed promotion for those failing to meet standards.

iv) Expanding training channels. Expand the workforce through "academic qualifications + training + social recruitment": encourage colleges and vocational schools to offer majors related to older adults services, forming a hierarchically connected system; and provide targeted training for rural laborers and guide them into the profession *via* subsidies; conduct standardized service training for institutional staff through "online + offline" models.

Additionally, attention to care workers' physical and mental health is needed to improve job satisfaction: establish regular counseling mechanisms such as quarterly group psychological counseling, and implement flexible work systems to relieve pressure. Incorporate mental health into assessments, improve occupational health safeguards including protective equipment and regular physical examinations, to prevent job burnout and talent loss.

In conclusion, systematically improving the development of the older adults care workforce through enhancing top-level design, optimizing incentives, upgrading training systems, expanding training channels, and focusing on care workers' well-being can adapt to the demand for older adults care services amid population aging.

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Review

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APOE4 reprograms microglial lipid metabolism in Alzheimer's disease: Mechanisms and therapeutic implications

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SUMMARY: The apolipoprotein E ε4 (*APOE ε4*) allele, the strongest genetic risk factor for late-onset Alzheimer's disease (AD), induces cell-type-specific disturbances in brain lipid metabolism. Although impacting astrocytes and neurons, its most pronounced effects occur in microglia, where it causes energy metabolism deficits and promotes the formation of lipid droplet-accumulating microglia, triggering a cascade of neurodegenerative responses. This review comprehensively examines how microglial APOE4-driven lipid metabolic dysregulation exacerbates neuroinflammation and compromises phagocytic capacity, particularly in the clearance of amyloid-β, phosphorylated-tau, and pathological synapses. Mechanistically, microglial APOE4 activates neuroinflammation *via* LilrB3-mediated type I interferon signaling and induces lipid metabolic imbalance through PU.1/NF-κB-driven transcriptional reprogramming and ER stress-SREBP2 activation. These disturbances exacerbate neuroinflammation, promote lipid droplet accumulation and cholesterol overload, impair lysosomal function, and ultimately compromise microglial phagocytosis. The resulting disruption of neuron-microglia interactions further amplifies neurotoxicity in AD. Furthermore, this review summarizes emerging therapeutic strategies targeting APOE4-related pathway in microglia. By synthesizing these insights, this review highlights the multifaceted role of microglial APOE4 in AD pathology, with particular emphasis on the central role of lipid metabolism dysregulation, and provides new intervention ideas for reducing its damage to brain function.

Keywords: Alzheimer's disease, apolipoprotein E4, microglia, lipid metabolism, neuroinflammation, phagocytosis

1. Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease — responsible for 50-70% of dementia cases worldwide — and is characterized primarily by progressive cognitive dysfunction (*1*). As the global population ages, the prevalence of AD continues to increase, imposing a substantial burden on affected individuals and health care systems (*2*). The primary pathological features of AD include the abnormal accumulation of extracellular amyloid-β (Aβ) and phosphorylated tau (p-tau) protein in neurons. Concurrently, inflammatory responses, synaptic dysfunction, and neuronal loss are significant characteristics that cannot be overlooked in AD. Notably, significant changes in brain lipid peroxidation levels can be observed in the early stage of AD. These metabolic disorders of lipid components are closely related to the core pathological mechanisms of AD, including Aβ deposition, p-tau, oxidative stress, and mitochondrial dysfunction (*3*). These findings underscore the critical role of lipid metabolism imbalance in AD pathogenesis and provide important directions for identifying AD-biomarkers and discovering novel therapeutic strategies through lipidomics research.

An estimated 60-80% of the susceptibility to AD can be attributed to genetic factors, with the apolipoprotein E ε4 *(APOE*ε4) allele recognized as the primary genetic risk factor for late-onset AD (*4-6*). *APOE*ε4 carriers exhibit a greater risk of developing AD. Specifically, individuals with a single *APOE*ε4 allele face an approximately 3- to 4-fold greater risk of developing AD than noncarriers do, while those with two *APOE*ε4 alleles have a 9- to 15-fold greater risk (*7*). In addition to exacerbating Aβ accumulation, tau hyperphosphorylation, and synaptic loss, APOE4 severely disrupts cerebral lipid metabolism and lipid transport, leading to cellular dysfunction, neuroinflammation activation, and myelin impairment — all of which are hallmarks of AD progression. Consequently, investigating APOE4-related lipid metabolism dysregulation provides critical insights into the pathogenesis and therapeutic development of AD (*8-11*).

Microglia are resident immune cells in the central nervous system (CNS) that primarily perform immunosurveillance, neurotrophic support, and plasticity functions in the brain (*12*). At the onset of AD, microglia

recognize and phagocytose Aβ to prevent its aggregation. However, in the later stage of AD, neuroinflammation induced by reactive microglia promotes the deposition of Aβ and the formation of neurofibrillary tangles (NFTs). Additionally, reactive microglia phagocytose synapses and disrupt neuronal communication in AD (*13*). APOE4-related lipid metabolism disorders can induce metabolic reprogramming of microglia into a proinflammatory state, impairing their phagocytic function (*14,15*). This review first provides an overview of APOE4, and then analyzes APOE4-related lipid metabolism disorders at the cellular level, with particular emphasis on its impact on microglial lipid metabolism and subsequent effects on phagocytic and secretory functions during AD progression. Finally, we discuss emerging therapeutic strategies targeting APOE4-microglia interactions, highlighting their potential to restore microglial homeostasis and mitigate AD pathogenesis.

2. Overview of APOE4

2.1. Structure and function of APOE4

The *APOE* gene is located on chromosome 19 and encodes a secreted glycoprotein consisting of 299 amino acids with a molecular weight of approximately 34 kDa. Human *APOE* is polymorphic and comprises three distinct alleles: ε2, ε3, and ε4. The amino acid positions of the three isoforms encoded by the *APOE* allele differ at positions 112 and 158: Cys112/Cys158 for APOE2, Cys112/Arg158 for APOE3, and Arg112/Arg158 for APOE4 (*16-18*) (Figure 1A). The different APOE isoforms differ in their oligomerization tendency, structural stability, and binding affinity to lipids, receptors, and Aβ peptides. Structurally, APOE4 is the least stable isoform. It adopts a folded intermediate state characterized by a core α-helical structure, increased β-lamellar structure, and an enlarged hydrodynamic radius, collectively resulting in a "molten globule" state (*19,20*). This semi-folded configuration enhances the interaction of APOE4 with larger lipid-rich particles and Aβ deposits in the brain. Simultaneously, this "molten globule" state promotes the aggregation of lipid-deficient APOE4, impairing its lipid transport capacity and facilitating Aβ accumulation (*21,22*). This phenomenon

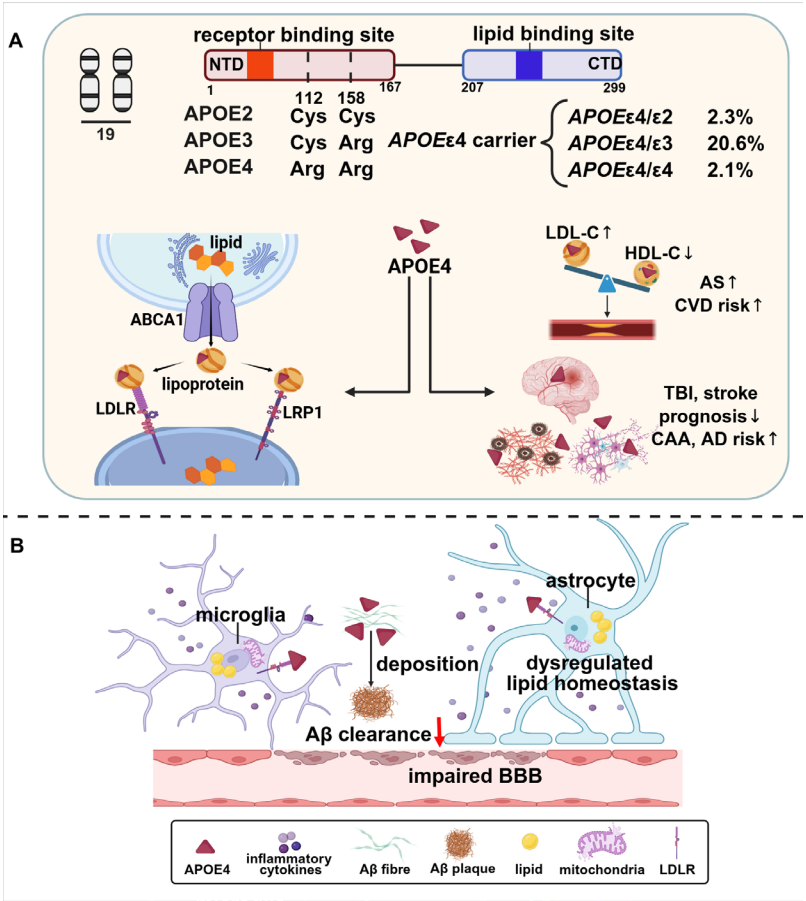

Figure 1. APOE4-mediated dysregulation and neurological implications. (A). Chromosomal location of the *APOE* gene and differences in different protein isoforms, the percentage of *APOEε4* gene carriers, and the association of APOE4 with multiple diseases. **(B)**. In AD, APOE4 promotes Aβ deposition and impairs Aβ clearance through receptor competition and BBB disruption. APOE4 drives astrocytes and microglia toward a pro-inflammatory phenotype and induces mitochondrial dysfunction. Importantly, APOE4 disrupts intracellular lipid metabolism, leading to pathological lipid droplet accumulation and accelerated AD progression. ABCA1, ATP-binding cassette transporters A1; LDLR, low-density lipoprotein receptor; LRP1, LDL receptor-related protein 1; AS, atherosclerosis; BBB, blood-brain barrier; CVD, cardiovascular disease; TBI, traumatic brain injury; CAA, cerebral amyloid angiopathy.

may represent a potential mechanism through which APOE4 contributes to the pathogenesis of AD.

Functionally, APOE participates in lipid metabolism and transport *via* lipoprotein particles. Lipidation occurs through two mechanisms: intracellular presecretory lipidation *via* the endoplasmic reticulum (ER)/Golgi pathway, and extracellular lipidation mediated by ATP-binding cassette transporter A1 (ABCA1) (*10,23,24*). ABCA1 is expressed in peripheral hepatocytes, macrophages, and intestinal epithelial cells to maintain systemic lipid homeostasis and in CNS glial cells and neurons to preserve lipid homeostasis in the brain, with its receptor proteins regulated by the retinoid X receptor (RXR) and liver X receptor (LXR) system (*25*). For lipid delivery, these lipoprotein particles facilitate intercellular lipid transport through receptor-mediated endocytosis involving low-density lipoprotein receptor (LDLR) and LDL receptor-related protein 1 (LRP1) (*26*). As an important lipid carrier, the APOE4 isoform has abnormal lipid metabolism properties, which leads to increased levels of low-density lipoprotein cholesterol (LDL-C) and decreased levels of high-density lipoprotein cholesterol (HDL-C), thus promoting atherosclerosis and increasing the risk of cardiovascular diseases (*19,27*). Moreover, *APOE*ε4 is an important risk factor for AD and cerebral amyloid angiopathy because it affects lipid homeostasis in the CNS, and *APOE*ε4 carriers exhibit poorer outcomes following stroke and traumatic brain injury, as well as accelerated motor progression and cognitive decline in Parkinson's disease patients (*28-31*) (Figure 1A). Overall, APOE4 drives multisystem pathologies through dysregulated lipid metabolism, contributing to cardiovascular diseases, neurodegenerative disorders, and poor neurological recovery, highlighting its central role in disease mechanisms.

2.2. APOE4 and AD

The *APOE*ε4 allele represents the strongest genetic risk factor for late-onset AD, with carriers facing an increased risk of developing AD and often experiencing an earlier age of onset (*5,6,16*). The carrier rate of *APOE*ε4 in the general population is approximately 23.9%, with 2.1% *APOE*ε4/ε4, 20.6% *APOE*ε3/ε4, and 2.3% *APOE*ε2/ε4 (*32*) (Figure 1A). Notably, nearly all *APOE*ε4 homozygote carriers display AD-related pathologic features, making it crucial to explore the relationship between *APOE*ε4 and the risk of developing AD (*33*). Epidemiological studies have demonstrated that the prevalence of *APOE*ε4-associated AD risk is associated with racial and sexual dimorphism, with East Asians having the highest susceptibility, followed by non-Hispanic Whites, while non-Hispanic Blacks and Hispanics have a lower risk, and female carriers face a significantly greater risk than males do (*34,35*). Additionally, genetic modifications complicate the relationship between the *APOE*ε4 allele and AD risk. For instance, both Klotho-VS heterozygotes and *APOE*ε4-R251G can attenuate the *APOE*ε4-associated AD risk (*36-38*). Specific single-nucleotide polymorphisms, including rs10553596 in the *CASP*7 gene and rs4934-A/A in the *SERPINA*39 gene, also reduce the high risk of AD in *APOE*ε4 heterozygotes (*39*). Strikingly, comorbidities such as atherosclerosis, peripheral vascular disease, and diabetes mellitus increase the risk of cognitive decline in *APOE*ε4 carriers (*40*), suggesting that managing cognition-related risk factors in *APOE*ε4 carriers may represent a potential therapeutic approach.

Extensive mechanistic investigations have substantiated the epidemiological evidence linking APOE4 to AD pathogenesis. In the amyloid pathway, APOE4 not only directly interacts with Aβ to promote its deposition in the CNS and accelerates the conversion of soluble Aβ to insoluble fibrils but also competitively binds to receptors such as LDLR, significantly inhibiting receptor-mediated Aβ clearance (*41-44*). APOE4-related blood-brain barrier (BBB) disruption also negatively affects Aβ clearance and precedes neuronal dysfunction, suggesting that vascular abnormalities may initiate neurodegeneration (*45,46*). In a nonamyloid-dependent pathway, APOE4 not only drives astrocytes and microglia toward a proinflammatory phenotype, impairing their immune function and exacerbating neuroinflammatory responses but also induces mitochondrial dysfunction, thereby impairing fatty acid oxidation (FAO) and disrupting the energy supply of the brain (*47-49*). Most importantly, APOE4 disrupts cholesterol and triglyceride transport and metabolism, altering cellular membrane lipid composition and inducing pathological lipid droplet (LD) accumulation (*50*). These lipid metabolic disturbances not only directly dysregulate Aβ metabolism but also impair endocytosis, lysosomal function, and brain energy homeostasis while promoting oxidative stress (*51,52*) (Figure 1B). In particular, an imbalance in cholesterol homeostasis can affect the formation of oligodendrocyte myelin, which in turn affects learning and memory ability (*53*). Although APOE4 critically contributes to AD progression through these multifaceted lipid metabolic disturbances, the underlying mechanisms exhibit cell-type specificity, which will be systematically examined in the following cellular-level analysis.

3. Lipid metabolic disturbances in diverse cell types mediated by APOE4

3.1. Astrocytes and neurons

Astrocytes serve as the primary source of APOE in the CNS, with its expression modulated by CCAAT/enhancer-binding protein β (C/EBPβ) and mitochondrial function (*54,55*). While astrocyte-derived APOE normally maintains lipid homeostasis, supports synaptic pruning, and preserves the integrity of the BBB, APOE4 astrocytes exacerbate neurodegenerative processes

through multiple synergistic pathways (*56,57*). Studies have shown that APOE4 astrocytes exhibit profound dysregulation of lipid metabolism, characterized by aberrant sterol regulatory element-binding protein 2 (SREBP2) activation, which increases de novo cholesterol synthesis despite lysosomal dysfunction-induced accumulation (*58*). This metabolic imbalance involves upregulated lipid metabolism genes but downregulated transport genes, potentially mediated by reduced peroxisome proliferator-activated receptor γ (PPARγ) expression (*9,56,59*). This pathological cholesterol accumulation disrupts lysosome-dependent mitophagy, leading to mitochondrial dysfunction and early AD energy deficits (*52*). In addition, APOE4 astrocytes also accumulate enlarged, oxidation-prone LDs enriched with unsaturated triglycerides while secreting poorly lipidated lipoproteins that inefficiently support neuronal lipid demands, which impairs synaptogenesis and neuronal viability (*60-62*). In addition to these lipid metabolic disorders, APOE4 astrocytes upregulate glypican-4 (GPC-4) expression, increase LRP1 membrane trafficking to promote tau propagation and hyperphosphorylation, and impair APOE4-mediated miRNA transfer to neurons, thereby disrupting neuronal metabolic and epigenetic regulation and ultimately contributing to synaptic dysfunction and memory deficits (*63,64*) (Figure 2A).

Neuronal APOE expression is increased during stress and aging, exerting early detrimental effects on synaptic function and neurodevelopment (*65,66*). At the metabolic level, APOE4 significantly interferes with neuronal function through lipid-dependent pathways. Excessive binding of APOE4 to LDLR leads to increased neuronal lipid uptake, resulting in lysosomal dysfunction, lipofuscin accumulation, and impaired autophagy, which subsequently triggers tau protein aggregation and brain cell death (*67*). Concurrently, APOE4-expressing neurons exhibit deficient fatty acid (FA) storage in LDs, leading to the pathological accumulation of free FAs and an increased risk of lipotoxicity (*61*). Although neighboring astrocytes take up these lipids, APOE4 impairs their transport and oxidation capacity, particularly in the hippocampus (*61,67*). Furthermore, APOE4 promotes ABCA1 degradation, reduces cholesterol efflux and activates mTORC1-mediated senescence pathways, ultimately impairing synaptic plasticity (*68*). Structurally,

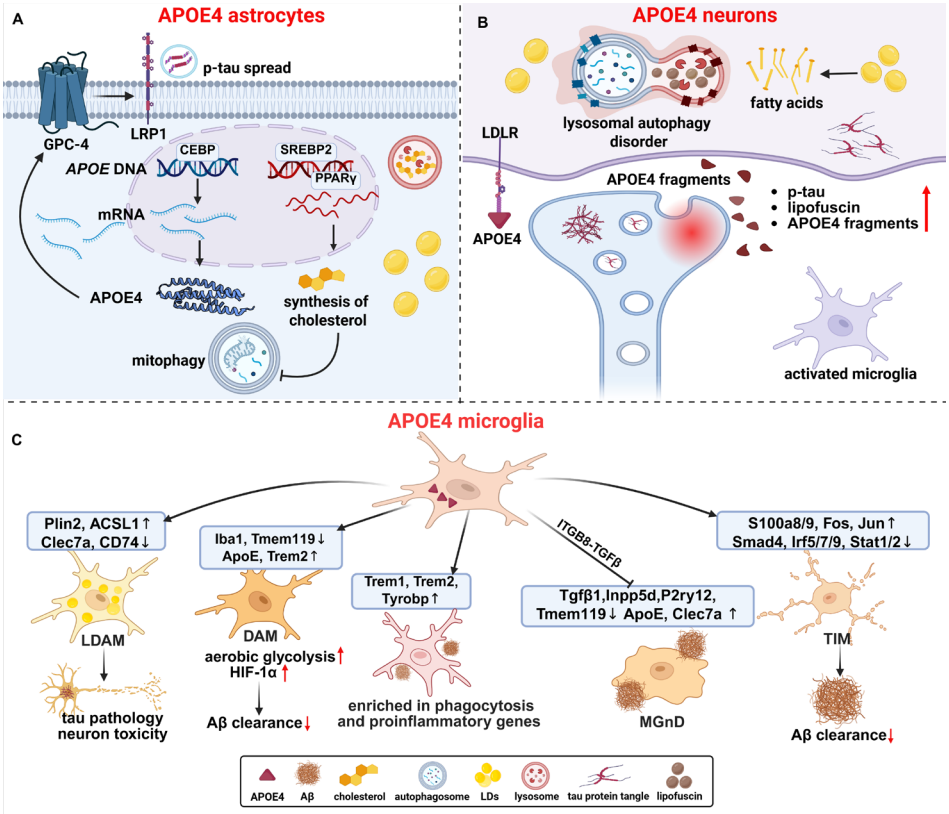

Figure 2. APOE4-mediated dysfunction of cellular lipid metabolism. (A). APOE4 astrocytes exhibit dysregulated lipid metabolism, characterized by SREBP2 activation, de novo cholesterol synthesis, and PPARγ suppression. Pathological cholesterol accumulation impairs mitophagy, leading to mitochondrial dysfunction. Additionally, APOE4 astrocytes specifically upregulates GPC-4, enhancing LRP1-mediated tau propagation. **(B).** APOE4 is hydrolyzed by neuron-specific proteases, producing neurotoxic fragments that exacerbate tau pathology and activate microglia. Metabolically, FAs are increased in APOE4 neurons, and hyperbinding of APOE4 to LDLR further increases lipid uptake in neurons, leading to lipid metabolism disorders, triggering lysosomal dysfunction, lipofuscin accumulation, and impaired autophagy-mediated tau protein aggregation. **C.** APOE4 microglia exhibit diverse phenotypic features, including LDAM, DAM, phagocytosis and pro-inflammatory phenotype, MGnD and TIM. SREBP2, sterol regulatory element-binding protein 2; GPC-4, glypican-4; FAs, fatty acids; LDAM, lipid-droplet-accumulating microglia; DAM, disease-associated microglia; MGnD, neurodegenerative microglia; TIM, terminally inflammatory microglia.

APOE4 undergoes neuron-specific proteolysis, generating neurotoxic fragments that destabilize the cytoskeleton and exacerbate tau pathology (*69*) (Figure 2B). APOE4 also selectively depletes GABAergic neurons, potentially disrupting neural network balance, while activating microglia through cell-nonautonomous mechanisms to amplify neuroinflammation (*70,71*). These findings establish that APOE4 links metabolic dysregulation to neurodegenerative processes by interfering with neuronal lipid homeostasis.

3.2. Microglia

Microglial APOE expression is significantly upregulated in response to injury and inflammation, demonstrating a close association with lipid metabolic dysregulation. Under physiological conditions, microglia maintain finely regulated lipid metabolic homeostasis, where the dynamic equilibrium between fatty acid synthesis (FAS) and FAO is essential for their immune surveillance functions (*15*). However, the *APOE*ε4 genotype disrupts this equilibrium, driving microglia toward a dysfunctional, proinflammatory lipid droplet-accumulating microglia (LDAM) phenotype (*72,73*). Specifically, APOE4 promotes de novo lipogenesis and LD accumulation by upregulating FAS while simultaneously suppressing autophagy-related genes to impair lipophagy (*73,74*). Additionally, APOE4 inhibits FAO *via* a dual mechanism — directly by inducing mitochondrial structural and functional damage that significantly reduces β-oxidation capacity, and indirectly by promoting microglial polarization toward a proinflammatory phenotype that downregulates FAO-related gene expression (*75,76*). This "enhanced synthesis-suppressed degradation" imbalance ultimately leads to the formation of pathological LDAM. In AD, microglia undergo dynamic lipid metabolic reprogramming. During the early stages, the triggering receptor expressed on myeloid cells 2 (TREM2)-APOE pathway mediates lipid uptake and FAO to support energy demands and facilitate Aβ clearance (*77*). However, chronic exposure to Aβ and tau pathology shifts the metabolism toward LD accumulation, resulting in LDAM (*73*). At the energy metabolism level, APOE4 microglia exhibit significant mitochondrial dysfunction, leading to reduced tricarboxylic acid cycle (TAC) efficiency and impaired FAO, in addition to hypoxia-inducible factor 1-α (HIF1α)-driven metabolic rewiring from oxidative phosphorylation to glycolysis (*78,79*). Intriguingly, a unique compensatory mechanism has been identified in microglia. In the context of AD, the expression of the glycolytic enzyme hexokinase 2 (HK2) is upregulated in microglia, and pharmacological inhibition of HK2 can subsequently activate lipoprotein lipase to increase lipid metabolism, thereby sustaining ATP production and promoting Aβ clearance (*80*). This glycolytic-lipid metabolic coupling appears to be microglia specific, as it has not been observed in other brain cells (*80*). These findings suggest that microglia respond to pathological stimuli through dynamic metabolic reprogramming mechanisms, but the *APOE*ε4 genotype drives microglia to a dysfunctional subtype by disrupting lipid metabolic balance and energy supply, ultimately exacerbating the neurodegenerative process.

As immune cells of the CNS, microglia can rapidly move and migrate extensively to perform immune surveillance and tissue repair functions. However, the *APOE*ε4 genotype significantly impairs these properties, resulting in reduced microglial mobility and reactivity, accompanied by marked morphological abnormalities that ultimately impair their immune surveillance and phagocytosis functions. Specifically, APOE4-expressing microglia exhibit irregular structural features, including enlarged cell bodies and nuclei, shortened processes, and a flattened, discoid shape (*9,81,82*). Advances in single-cell sequencing technology have further revealed that microglia exhibit different subtypes, mediated by the reprogramming of their cellular metabolism during development, growth, and disease. Among these, the LDAM subtype emerges as a canonical APOE4-driven pathological subtype characterized by dysregulated lipid metabolism, a proinflammatory state, and impaired phagocytic function (*72,73,81*). Disease-associated microglia (DAMs) exhibit high expression levels of genes involved in lipid metabolism and phagocytosis. APOE4-expressing microglia exhibit metabolic features consistent with those of DAMs, with increased aerobic glycolysis and Hif1a expression but impaired Aβ uptake (*9,78*). Moreover, a subset of microglia enriched in phagocytic and proinflammatory genes has been identified in *APOE*ε4 carriers, clustering around neuroinflammatory plaques and driving the conversion of microglia to phagocytic and proinflammatory phenotypes *via* the APOE–TREM2–TYROBP axis (*83*). In neurodegenerative diseases, the TREM2–APOE pathway mediates the transition of microglia into neurodegenerative microglia (MGnD), which exert a neuroprotective effect by eliminating apoptotic neurons (*84*). However, APOE4 exacerbates neurodegeneration by activating ITGB8–TGFβ signaling, upregulating the expression of homeostatic checkpoint molecules such as Inpp5d, and inhibiting MGnD function (*85*). Additionally, terminal inflammatory microglia (TIMs), another APOE4-associated exhausted subtype, display profound Aβ clearance deficits in both AD patients and mouse models (*86*) (Figure 2C). In conclusion, these findings demonstrate that APOE4 exacerbates neurodegenerative progression through multiple mechanisms, orchestrating the production of diverse subtypes of dysfunctional microglia that exhibit metabolic disturbances, inflammatory dysregulation, and phagocytic impairment.

4. Microglial APOE4 and AD

4.1. APOE4 in microglia exacerbates abnormal lipid metabolism

AD was initially described by the identification of numerous glial cells displaying lipid vacuoles in the brains of patients, highlighting the significant role of abnormal lipid metabolism in glial cells in the pathogenesis of AD (*87*). In particular, abnormal lipid metabolism in microglia not only exacerbates Aβ and tau pathology but is also directly associated with cognitive impairment in AD. Recent studies have shown that APOE4 induces the phosphorylation of eIF2α in microglia, activates the integrated stress response, and promotes the release of harmful lipids and synaptic loss (*88*).

4.1.1. POE4 in microglia and intracellular lipid droplet accumulation

The critical metabolic shift in the formation of microglial LDs involves a decrease in free FAs and an increase in triglycerides (*89*). This shift represents a defensive response that maintains lipid homeostasis and neutralizes lipid-mediated neurotoxicity by buffering excess free FAs and cholesterol. However, the lipid homeostasis of APOE4 microglia is often disrupted. Research has shown that *APOE*ε4 carriers and their induced pluripotent stem cell (iPSC)-derived microglia tend to accumulate LDs. This phenomenon may be linked to the stress-related responses promoted by microglial APOE4, which downregulates complement and lysosomal pathways, while excessive oxidative stress significantly contributes to LD accumulation (*81*). Furthermore, the binding of fibrillar Aβ to TREM2 receptors on the surface of microglia activates the PI3K–Akt–mTOR signaling pathway, leading to the overexpression of LD-related genes, including *ACSL*1 and *PLIN*2. This overexpression enhances triglyceride synthesis and promotes LD formation, especially in patients with AD carrying *APOE*ε4/ε4 (*73,90*). Notably, the number of LDs is negatively correlated with cognitive function but positively correlated with the levels of Aβ plaques and tau pathology, suggesting a role for LDs in AD progression. ATAC-seq and RNA-seq analyses revealed that enhancer regions in LD-rich APOE4 microglia are highly enriched in protein upstream of .1 (PU.1) and nuclear factor kappa-B (NF-κB) family factors. PU.1 regulates genes involved in LD formation, while NF-κB increases proinflammatory gene expression and induces the transformation of microglia into a proinflammatory phenotype involved in neuroinflammation, disrupting the neural microenvironment (*73*). LDAM often accumulate near Aβ plaques, impair lysosomal function, affect phagocytosis, and may accelerate the spread of plaques. Moreover, excessive proinflammatory cytokines and ROS are released, promoting neuronal lipogenesis. Abnormally increased lipids can be transported to microglia *via* APOE to synthesize LDs, thus forming a vicious cycle (*72*) (Figure 3). Surprisingly, in AD model mice, the accumulation of human iPSC-derived microglial LDs largely depends on microglial reactivity and proximity to plaques, which are impaired by the TREM2-R47H mutation. Specifically, TREM2 R47H-mutant microglia exhibited reduced LD accumulation *in vivo*, decreased plaque reactivity, and decreased plaque-associated APOE secretion, whereas the same mutation exacerbated LD accumulation *in vitro*, highlighting the critical regulatory role of the environmental context in microglial function (*91,92*).

These findings highlight how APOE4 disrupts the delicate balance of microglial lipid metabolism, transforming a potentially protective LD formation process into a maladaptive response that promotes AD progression through impaired phagocytosis, sustained neuroinflammation, and the creation of a neurotoxic lipid microenvironment. The microenvironment-dependent phenotypes observed in chimeric models further emphasize the critical interplay between cell-intrinsic metabolic reprogramming and the pathological brain milieu in shaping microglial dysfunction (*91*).

4.1.2. APOE4 microglia and abnormal cholesterol metabolism

Cholesterol is an indispensable lipid in cellular membranes and is essential for maintaining membrane fluidity and integrity (*93*). Numerous analyses of clinical data have revealed abnormal cholesterol accumulation in the cores of mature Aβ plaques, with elevated cholesterol levels in the brain often associated with AD-related cognitive decline and exacerbation of clinical symptoms. In particular, dysregulated cholesterol metabolism in microglia is thought to be a major driver of senescence pathologies in AD (*94,95*). APOE, a cholesterol transport protein, is vital for the survival and phagocytic function of microglia. However, microglial APOE4 disrupts cholesterol homeostasis and alters cholesterol transport-related signaling pathways, impairing myelination and subsequently affecting cognitive function (*51,53*). Evidence indicates that cholesterol overload occurs in APOE4 microglia, potentially because of increased synthesis driven by the stress–ER Ca^{2+}–SREBP2 pathway. Specifically, microglial APOE4 induces ER stress, leading to Ca^{2+} depletion in the ER and activation of the SREBP2 transcription factor. This activation promotes the transcription of key genes, such as *HMGCR* and *SQLE*, which regulate the cholesterol biosynthesis pathway (*96*). Furthermore, microglial APOE4 may impair ABCA1 recycling, lysosomal function, and cholesterol efflux and metabolism, leading to the accumulation of intracellular cholesterol that ultimately reduces the capacity for Aβ degradation (*51,97*) (Figure 3). In addition to Aβ clearance, cholesterol may significantly influence Aβ plaque formation because of its uneven distribution in the cell membrane, where it

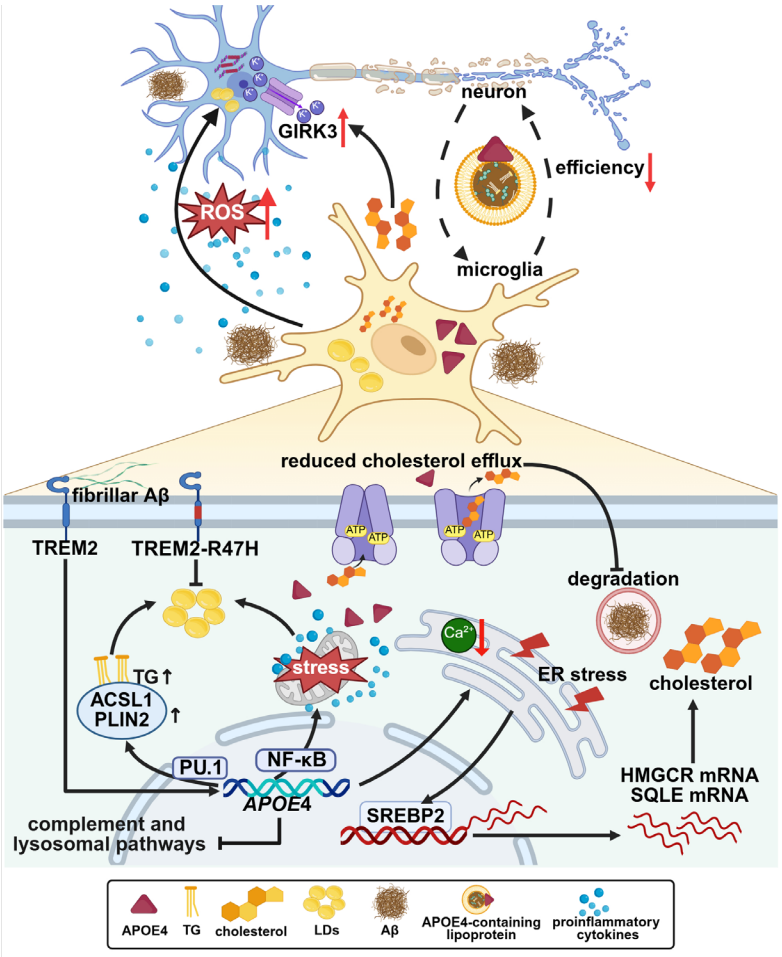

Figure 3. Microglial APOE4-mediated dysregulation of lipid metabolism in AD. Microglial APOE4-mediated upregulation of triglyceride synthesis exacerbates the accumulation of LDs under Aβ stimulation, whereas TREM2-R47H reduces intracellular LDs accumulation. In addition, microglial APOE4 promotes cholesterol synthesis *via* the ER stress-ER Ca^{2+}-SREBP2 pathway and impairs cholesterol efflux, affecting Aβ degradation in lysosomes. The accumulation of extracellular cholesterol increased GIRK3 and decreased neuronal excitability. Microglial APOE4 also decreased lipid transport efficiency and disrupted lipid metabolic coupling in neurons. Ultimately, phagocytosis-impaired APOE4 microglia release pro-inflammatory cytokines and ROS that promote neuronal lipogenesis and translocation to microglia, creating a vicious cycle. ACSL, acyl-CoA synthetase long-chain; ER, endoplasmic reticulum; GIRK3, G protein-gated inwardly rectifying potassium channel 3; ROS, reactive oxygen species; LDs, lipid droplets; TREM2, triggering receptor expressed on myeloid cells 2.

aggregates with sphingolipids and scaffolding proteins to form lipid rafts. These rafts are involved in the cleavage of amyloid precursor proteins and the generation of Aβ by α-, β-, and γ-secretases. Disruption of cholesterol homeostasis can lead to abnormal accumulation and release of Aβ, promoting plaque formation (*98,99*).

Recent studies have demonstrated that using LXR agonists, overexpressing ABCA1, or activating TRPV1 can increase cholesterol efflux and reduce the accumulation of cholesteryl esters in microglia, thereby ameliorating APOE4-related neurodegeneration (*51,96,100*). These findings suggest that APOE4 disrupts cholesterol homeostasis in microglia through multiple mechanisms, including enhanced cholesterol biosynthesis *via* the ER stress pathway and impaired efflux *via* ABCA1 dysfunction. The resulting cholesterol overload not only impairs Aβ clearance and promotes plaque formation, but also contributes to broader neurodegenerative processes by disrupting myelin integrity and synaptic function. Importantly, therapeutic strategies targeting cholesterol metabolism have shown promise in alleviating APOE4-driven pathology, highlighting the central role of cholesterol dysregulation in AD pathogenesis (*51,68,96,100*).

4.1.3. APOE4-mediated dysregulation of lipid metabolism in microglia disrupts communication between neurons and microglia

Microglia serve as sentinels in the neural network, and their interactions with neurons are regulated by a complex array of intercellular signaling mechanisms, including purinergic signaling, cytokines, neurotransmitters, and neuropeptides (*101,102*). However, APOE4 alters purinergic signaling and lipid metabolism in microglia, which in turn affects their communication with neurons. It has been demonstrated that APOE4 induces a pronounced accumulation of LDs in microglia, which not only

impairs their phagocytosis and clearance of pathological proteins but also disrupts their interactions with neurons (*8,72,103*). Mechanistic studies revealed that APOE4 drives these pathological processes through multiple convergent pathways. It compromises the lipid uptake efficiency of microglia, leading to extracellular lipid accumulation and enhanced proinflammatory signaling, ultimately attenuating their ability to monitor neuronal network activity — a phenomenon closely aligned with the aberrant neural network activity observed in AD patients. In addition, APOE4 shifts microglial energy metabolism from oxidative phosphorylation toward glycolysis, further exacerbating intracellular lipid accumulation (*103,104*). This metabolic dysregulation triggers a cascade of pathological consequences. The most immediate manifestation is impaired lipid reuptake by APOE4 microglia, resulting in extracellular cholesterol accumulation that induces significant hyperpolarization of neuronal resting membrane potentials and upregulation of G protein-coupled inwardly rectifying potassium channels (GIRK3), collectively reducing neuronal excitability (*103*) (Figure 3). More critically, LDAM secrete toxic factors that not only promote abnormal p-tau deposition in neurons but also activate apoptotic pathways (*73*). Concurrently, proinflammatory cytokines released by LDAM drive intracellular LD accumulation in neurons, exacerbating neuronal damage. Interestingly, neurons are not passive recipients but actively regulate microglial lipid metabolism through AMPK-mediated suppression of lipogenesis and activation of lipophagy, thereby modulating lipid flux to microglia and demonstrating a sophisticated bidirectional regulatory mechanism (*105*).

These findings provide novel insights into the pathogenesis of neurodegenerative diseases. APOE4 disrupts metabolic coupling between neurons and microglia by impairing microglial lipid transport capacity, which is characterized by decreased APOE-containing lipoprotein particles and reduced lipidation levels, ultimately leading to neural network dysfunction (*106*). Building upon this understanding, current research is focused on developing innovative therapeutic strategies to increase APOE4-mediated lipid transport efficiency. By restoring metabolic homeostasis and restoring neural network stability, these interventions represent promising avenues for AD treatment.

4.2. Microglial APOE4 promotes neuroinflammation

Neuronal death caused by neuroinflammation in AD is far greater than that caused by Aβ plaques and NFTs, establishing neuroinflammation as a hallmark pathological feature of AD (*107-110*). Microglia, the central orchestrators of this inflammatory cascade, release a plethora of cytokines, including tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β), which are crucial for modulating the inflammatory cascade. Importantly, microglial APOE4 has a bidirectional interaction between lipid metabolism disorders and neuroinflammation: it both directly activates inflammatory pathways and aggravates neuroinflammation by disrupting lipid homeostasis (*72,73,75,111*).

First, microglial APOE4 directly activates inflammatory pathways through multiple mechanisms. Zhou *et al.* reported that APOE4 binds to LilrB3 on the surface of microglia to upregulate the expression of relevant type I interferon-stimulating genes, including *IFITM*3, *BST*2, *MX*1, *ISG*15, and *STAT*1. This transition drives microglia to enter a proinflammatory state, hinders their phagocytic function, and contributes to Aβ deposition (*75*). In addition to this receptor-mediated pathway, APOE4 also intrinsically primes microglia toward inflammation. In the basal state, APOE4-expressing microglia exhibit an obvious proinflammatory effect, which manifests as increased activation of the NLRP3 inflammasome and excessive production of reactive oxygen species (ROS), leading to cellular immune dysfunction. This proinflammatory phenotype is further exacerbated by immune stimulation such as lipopolysaccharide (LPS) and interferon-gamma (IFN-γ), which promote the secretion of inflammatory factors such as TNF-α, IL-1β, NOS2 and MCP1 in large amounts, especially in female mice. More importantly, microglial $nAPOE_{41-151}$ significantly increases the expression of the proinflammatory cytokine TNF-α by inhibiting Cxorf56, thus leading to the formation of a full-spectrum inflammatory amplifying pathway from the basal state to the immune-activated state (*112-114*). All of the above findings indicate that microglial APOE4 activates neuroinflammation and affects microglial function. Second, APOE4-induced lipid dysregulation further amplifies this inflammatory response. Metabolic reprogramming of APOE4-expressing microglia enhances glycolysis, inhibits TAC, and directs carbon flux toward lipid synthesis (*78*). This results in the accumulation of pathological LDs and increased release of proinflammatory lipid mediators, including prostaglandins and arachidonic acid metabolites, activating neuroinflammatory pathways (*78*). Moreover, lipid peroxides and cholesterol accumulated by APOE4 microglia activate the NF-κB signaling axis, creating a self-reinforcing cycle of cytokine production and metabolic dysfunction (*73*). On the other hand, accumulated lipids enhance Major Histocompatibility Complex Class II (MHC-II)-dependent antigen presentation, thereby hyperactivating T cells and contributing to neuroinflammation (*100*). It has also been reported that APOE4 microglia secrete more oxysterol 25-hydroxycholesterol (25-HC) and IL-1β following LPS treatment, with 25-HC further significantly increasing IL-1β secretion; these findings highlight a lipid-driven mechanism through which APOE4 sustains chronic neuroinflammation (*111*) (Figure 4A). Collectively, APOE4 microglia-mediated lipid metabolism disturbances trigger cell membrane dysfunction

and the release of inflammatory mediators, which activate microglia while impairing their Aβ clearance capacity, ultimately promoting neuronal apoptosis. The accumulation of Aβ deposits and inflammation further exacerbate lipid metabolic dysregulation, creating a spatially specific self-reinforcing cycle. This bidirectional crosstalk mechanism reveals how APOE4 promotes neurodegenerative progression through "metabolism-inflammation" interplay.

Notably, therapeutic strategies targeting this lipid-inflammation axis have shown promising neuroprotective effects. Pharmacological agents such as acyl-CoA cholesterol Acyltransferase (ACAT) inhibitors demonstrate dual functionality by enhancing cholesterol efflux while concurrently suppressing NF-κB-mediated cytokine release (*115*). This metabolic reprogramming attenuates both neuroinflammation and lipid accumulation, effectively breaking the vicious cycle that drives disease progression.

4.3. Microglial APOE4 inhibits the clearance of pathological proteins

Microglial phagocytosis plays a pivotal role in maintaining CNS homeostasis by eliminating neurotoxic substances, including Aβ and p-tau, and participating in neural circuit remodeling. However, the *APOE*ε4 genotype substantially impairs this critical function through multifaceted mechanisms during AD pathogenesis. Under physiological conditions, the TREM2-dependent lipid metabolic network coordinates cholesterol efflux through the LXR/PPARγ pathway, maintains phago-lysosomal cycling, and activates microglia to phagocytose diffuse Aβ, compressing it into dense Aβ plaques that are less toxic and prevent its further spread in the brain (*44,116,117*).

The presence of APOE4 disrupts this sophisticated regulation through several interconnected pathways. It induces mitochondrial dysfunction while simultaneously compromising pseudopod extension capacity because of altered membrane fluidity from abnormal lipid accumulation, coupled with downregulated TREM2 expression, collectively impairing phagocytic capacity (*74,118*). Furthermore, APOE4 increases the likelihood of forming fibrillar aggregates within microglia that serve as nucleation sites for Aβ plaque formation and specifically suppresses $Aβ_{42}$ clearance by disrupting ITGB8–TGFβ signaling, as demonstrated by animal studies showing that selective ablation of microglial APOE4 expression restores their phagocytic function and markedly reduces the plaque burden (*42,85*). Interestingly, in female AD patients carrying *APOE*ε4, APOE4-expressing neutrophils upregulate the expression of IL-17F, which interacts with microglial IL-17RA to disrupt Aβ clearance, ultimately impacting cognitive function. Interrupting the IL-17F/IL-17RA signaling pathway ameliorates cognitive deficits and reduces Aβ deposition in AD (*119*) (Figure 4B). These findings systematically elucidate how APOE4 synergistically impairs microglial phagocytosis through multiple mechanisms such as lipid metabolism disorders to affect Aβ pathology.

Microglial phagocytosis also contributes to the propagation of tau pathology. In general, reactive microglia exert neuroprotective effects by actively engulfing tau proteins and tau-laden synapses and neurons for clearance (*120*). However, APOE4 fundamentally alters this homeostatic mechanism by

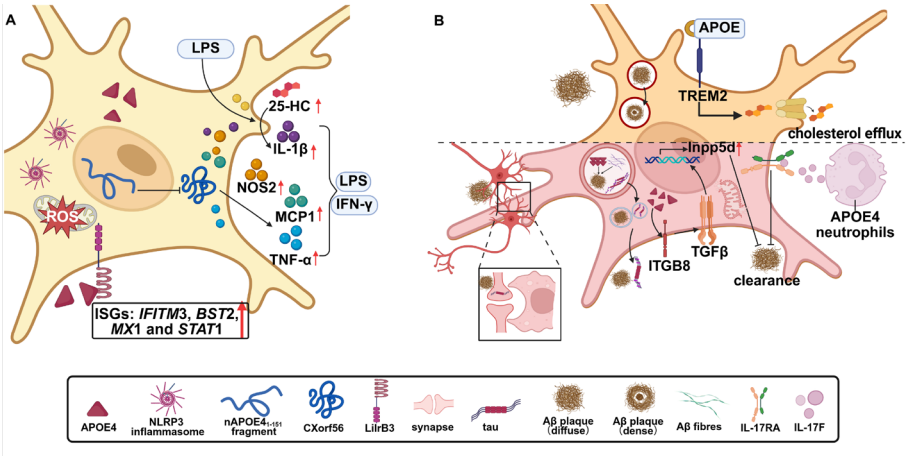

Figure 4. Microglial APOE4 promotes neuroinflammation and inhibits the clearance of pathological proteins in AD. (A). Microglial APOE4 promotes neuroinflammation by interacting with LilrB3 to upregulate ISGs, or inhibiting Cxorf56 to enhance TNF-α secretion in AD. In addition, NLRP3 inflammasomes and ROS were increased in APOE4 microglia, and more inflammatory factors were secreted in response to LPS and IFN-γ stimulation, further confirming the involvement of APOE4 in microglia-mediated neuroinflammation. **(B)**. Upper part: APOE accumulates around Aβ plaques, compacting diffuse Aβ into less toxic core plaques and restricting their dissemination within the brain. Lower part: microglial APOE4 inhibits Aβ clearance, promotes Aβ plaque formation, reduces tau degradation, and facilitates tau spread *via* exosomes. Meanwhile, APOE4 microglia enhance synaptic phagocytosis near Aβ plaques, correlating with cognitive decline. ISGs, interferon-stimulating genes; LPS, lipopolysaccharide; IFN-γ, interferon-gamma.

initiating a cascade of pathological events. APOE4-driven lipid metabolic dysregulation induces intracellular LD accumulation, which compromises plasma membrane integrity and severely impairs endosomal–lysosomal system functionality (*15,68,121*). This organelle dysfunction directly attenuates the processing and degradation capacity of tau proteins, leading to abnormal intracellular accumulation of p-tau (*51*). The accumulated pathological p-tau subsequently undergoes exosome-mediated release, thereby facilitating its intercellular propagation (*51,122*). Neuroimaging studies have consistently demonstrated significantly enhanced p-tau accumulation in APOE4 carriers, while the ameliorative effects of the APOE4-R136S homozygous mutation on tau pathology provide compelling genetic evidence for the pivotal role of APOE4 in regulating tau protein metabolism (*123,124*). In particular, aggregates of Aβ and tau near synapses have been shown to recruit microglia to phagocytose pathological synapses, thereby exacerbating synaptic dysfunction in AD. Increased phagocytosis of synapses by microglia was observed in the brains of AD patients carrying *APOE*ε4, which was particularly pronounced near Aβ plaques (*125,126*) (Figure 4B). Reactivation of transient receptor potential vanilloid 1 (TRPV1) has been shown to ameliorate cerebral lipid metabolic dysregulation, reduce LD accumulation, and attenuate microglial synaptic pruning, thereby ameliorating tau pathology and memory impairment (*96,100*). These findings reveal that APOE4 not only aggravates the impairment of Aβ/tau clearance, but also actively promotes pathological protein propagation and synaptic damage through dysregulation of the metabolism–phagocytosis pathway, suggesting that targeting the lipid metabolic reprogramming of APOE4 microglia may be a key strategy to attenuate AD progression.

APOE4 exacerbates AD progression through multifaceted mechanisms that disrupt microglial lipid homeostasis, thereby affecting neuroinflammation and the clearance of pathological proteins (*127,128*). APOE4-induced lipid metabolic dysregulation and mitochondrial dysfunction impair lysosomal degradation capacity, significantly compromising the clearance of Aβ and tau aggregates by microglia (*74*). Concurrently, aberrant lipid metabolism activates inflammatory signaling pathways, promoting excessive secretion of proinflammatory factors and establishing a persistent neuroinflammatory microenvironment. This inflammatory cascade further disrupts microglial function, ultimately resulting in neuronal death. These pathological alterations mutually reinforce each other, establishing a vicious cycle of "lipid metabolic dysregulation–neuroinflammation–functional impairment" (*14,15,72,73,78,100,111*). Consequently, therapeutic strategies targeting APOE4-mediated microglial lipid metabolic pathways, simultaneously suppressing inflammatory responses and restoring phagocytic function, may offer novel intervention approaches for AD treatment.

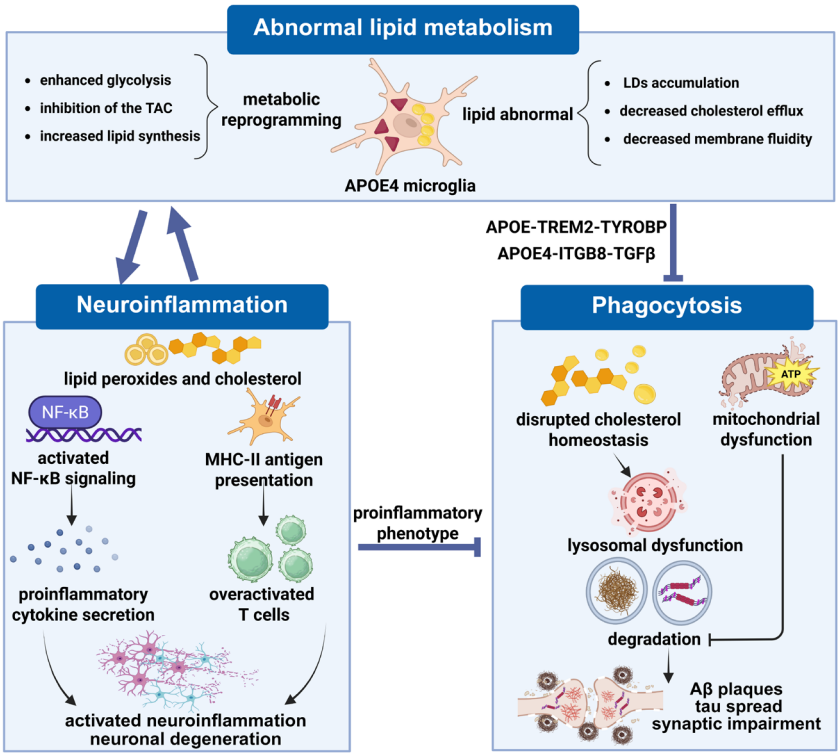

Figure 5. Summary of the role of microglial APOE4 in AD. APOE4-driven abnormal lipid metabolism of microglia is the core, which further aggravates neuroinflammation, eventually affects the immune phagocytosis of microglia, and accelerates the pathological process of AD.

5. Promising therapeutic strategies targeting microglial APOE4

Despite decades of research into treatments for AD, the therapeutic landscape remains limited. This review summarizes advances in microglia- and APOE4-related AD therapy (Table 1). In terms of metabolic regulation, lipid metabolism disorders can be alleviated by inhibition of cholesterol synthesis by TRPV1, promotion of cholesterol efflux by an LXR agonist (CS-6253), or enhancement of APOE4 lipidation by an RXR agonist (bexarotene) (*96,100,129-131*). Notably, pharmacological inhibition of diacylglycerol O-acyltransferase 2 (DGAT2) in microglia has been demonstrated to suppress triglyceride biosynthesis and subsequent LD accumulation, whereas genetic ablation of perilipin 2 (PLIN2) promotes LD degradation and attenuates neuroinflammation (*132,133*). These results suggest that targeting lipid metabolism is a promising therapeutic strategy for ameliorating pathological progression in AD. In the context of neuroinflammation, dimethyl malonate (DMM) reduces HIF1α expression in the microglia of AD model mice, promotes an anti-inflammatory phenotype, and attenuates neuroinflammation (*134*). Given that microglial APOE4 is associated with the upregulation of the NLRP3 inflammasome, modulation of the NLRP3 inflammasome is also a viable option to mitigate neuroinflammation (*114*). NLRP3 inhibitors, such as JC-124, dihydromyricetin (DHM), DAPPD, and dapansutrile (OLT1177), have shown potential in curbing neuroinflammation and enhancing Aβ clearance (*135-138*). Additionally, the ubiquitin ligase COP1 is another target for AD therapy because it modulates CEBP levels and attenuates proinflammatory gene expression in microglia (*139*). Strikingly, traditional herbal compounds, such as resveratrol and curcumin, have demonstrated the ability to inhibit microglia-associated neuroinflammation as potential therapeutic agents (*140-142*). The interaction between lipid metabolism and neuroinflammation is ultimately reflected in microglial dysfunction. For example, rutin sodium (NaR) and the 5-HT2A receptor antagonist desloratadine increase the expression of phagocytic receptors on the surface of microglia, and NaR promotes a shift in oxidative phosphorylation to generate the ATP required for efficient Aβ clearance (*143,144*). Furthermore, the AMPKα1 activator DW14006, and the TREM2 activator AL002c (NCT03635047, NCT04592874) have been shown to increase microglial phagocytosis of Aβ (*145-148*). Although some progress has been made in the study of microglial dysfunction as a therapeutic target for AD in animal models and at the cellular level, translating these findings into effective therapies in humans remains challenging.

Therapeutic strategies targeting the *APOE*ε4 allele have also been important focuses in AD research. Studies have reported that small-molecule mimetics such as $A\beta_{12\text{-}28}$p and the APOE mimic CN-105 reduce Aβ plaques and tau pathology by disrupting the interaction between APOE4 and Aβ (*149-151*). Recent research underscores the significant benefits of reducing APOE4 levels in AD. In a mouse model expressing human APOE4, immunotherapy with the anti-human APOE antibody HAE-4 has been shown to decrease the number of Aβ plaques and tau protein while inhibiting the expression of proinflammatory genes (*152,153*). Moreover, another promising approach involves the delivery of the human *APOE*ε2 gene *via* adeno-associated virus (AAV), which has been shown to prevent or even reverse the deleterious effects of APOE4 on brain amyloid pathology, with intracisternal delivery being the most effective method (*154,155*). LX1001, a drug targeting the *APOE*ε4 allele, has recently completed testing in phase I/II clinical trials. They have reported positive results regarding a dose-dependent increase in APOE2 protein expression and reductions in disease-associated tau protein biomarkers (*156,157*). The advent of CRISPR-Cas9 gene editing technology offers the potential to convert APOE4 to other isoforms, although this approach is accompanied by technical, ethical, and safety challenges (*9,158*).

Despite growing interest in microglial dysfunction and APOE4 as therapeutic targets for AD, no effective drugs currently exist to specifically correct APOE4-driven lipid metabolic abnormalities in microglia. Emerging evidence suggests that APOE4 exerts cell-type-specific pathogenic effects and that intervening with APOE4 in specific cell types can yield more precise results while alleviating the potentially toxic side effects associated with full-scale interventions targeting APOE4 (*159*). The critical role of microglial APOE4 in AD underscores its significance as a research focus, and future studies may provide breakthroughs in the treatment of AD.

6. Conclusion

AD is a progressive neurodegenerative disorder with complex pathogenic mechanisms. The *APOEε4* allele, the most significant genetic risk factor for AD, primarily mediates its pathological effects through microglial dysfunction, in which dysregulated lipid metabolism emerges as a pivotal pathogenic driver. Increasing evidence indicates that *APOEε4* disrupts microglial lipid homeostasis by impairing cholesterol efflux and promoting excessive LD formation, consequently (1) inducing proinflammatory cytokine secretion to activate microglia and aggravate neuroinflammation; (2) impairing phagocytic function by hindering energy metabolism, membrane fluidity and lysosomal activity; and (3) disrupting neuron–microglia crosstalk through lipid-mediated signaling pathways. The activation of neuroinflammation further aggravates abnormal lipid metabolism and affects the immune function of microglia. This pathogenic triad — lipid dysregulation,

Table 1. Promising therapeutic strategies targeting microglial APOE4 in Alzheimer's disease

Target Pathway	Therapeutic Agent	Mechanisms	Effects Observed	Note	*Ref.*
Lipid Metabolism	TRPV1/capsaicin	Inhibits SREBP-2, enhances autophagy activity	Alleviates cholesterol biosynthesis in APOE4 microglia; reduces APOE4 microglial phagocytosis of synapses	Specific to APOE4 microglia	(*96,100*)
	LXR agonist (GW3965), CS-6253	Increases cholesterol efflux in glial cells	Reduces lipid accumulation in glial cells; attenuates tau pathology	Specific to APOE4 microglia	(*51,129,131*)
	Bexarotene	Increases APOE4 lipidation	Reduces Aβ and p-tau accumulation; reverses cognitive and neuronal impairments	Specific to APOE4 Clinical trial: NCT01782742	(*130*)
Neuro-inflammation	JC-124, Dihydromyricetin, DAPPD, Dapansutrile (OLT1177)	NLRP3 inflammasome inhibitor	Suppresses neuroinflammation; promotes Aβ clearance; attenuates cognitive deficits	Targeting the NLRP3 inflammasome	(*135-138*)
	Ubiquitin ligase COP1	Modulates C/EBP levels and inhibits pro-inflammatory gene expression in microglia	Reduces microglial activation and neurotoxicity; suppresses neuroinflammation	Specific to microglia	(*139*)
	Resveratrol, Curcumin	Traditional Chinese Herbal medicine	Reduces neuroinflammation; neuroprotective	Specific to microglia	(*140-142*)
	Dimethyl Malonate	Inhibits succinate dehydrogenase (SDH) and HIF-1α expression in microglia	Enhances mitochondrial function; suppresses neuroinflammation	Specific to microglia	(*134*)
Phagocytosis	Sodium Rutin (NaR), Desloratadine	Increase the expression of phagocytic receptors in microglia	Enhances microglial phagocytosis of Aβ	Specific to microglia	(*143,144*)
	DW14006	Upregulates CD36 and modulates AMPKα1/IκB/NFκB signaling in microglia	Enhances microglial phagocytosis of Aβ; suppresses neuroinflammation	Specific to microglia	(*145*)
	AL002c	TREM2 activator	Reduces Aβ plaques and neurite dystrophy; tempers microglial inflammatory response	phase I clinical trial: NCT03635047; phase II clinical trial: NCT04592874	(*146-148*)
APOE4	Aβ12-28p, CN-105	disrupt the interaction between APOE and Aβ	Reduces Aβ pathology	Specific to APOE4	(*149-151*)
	HAE-4	Anti-human APOE4 antibody	Reduces Aβ plaques; inhibits tau propagation and neuritic dystrophy	Specific to APOE4	(*152,153*)
	LX1001	Increases APOE2 levels in the brain of APOE4 homozygous patients *via* AAV	Ameliorates tau pathology	Clinical trial: NCT03634007	(*156,157*)
	CRISPR-Cas9	Gene editing for APOE modification	Potential to convert APOE4 to other isoforms	technical, ethical, and safety challenges	(*158*)

sustained neuroinflammation and impaired phagocytosis — forms a self-perpetuating cycle that exacerbates AD progression. Current therapeutic strategies for this axis include restoring the homeostasis of lipid metabolism in microglia, reducing neuroinflammation, enhancing immune phagocytosis by microglia, and reducing the expression of APOE4. Future research should aim to elucidate the molecular mechanisms underlying APOE4-mediated lipid metabolism disorders in microglia, develop lipidomic signatures as predictive biomarkers for APOE4-targeted interventions, and design integrated treatment approaches that synergistically address multiple pathological cascades in AD.

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Original Article

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Exploring the pathway of a social network in promoting the individual performance of core members of social organizations caring for the elderly: A moderated mediation model of social support and self-efficacy

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SUMMARY: The high performance of core members of social organizations (SOs) caring for the elderly can enhance the quality of management and services, thereby improving the life satisfaction of older adults residing there. However, the factors influencing the performance of core members and their pathways remain unclear. This research seeks to uncover how social support mediates and self-efficacy moderates the association between a social network and individual performance of core members of SOs caring for the elderly. A cross-sectional survey was conducted from June to August 2023 in Shanghai, China, and data on participants' demographics, social network, social support, individual performance, and self-efficacy were collected. Hierarchical stepwise regression, bootstrap analysis, and simple slope method analysis were used to test potential mediating and moderating effects. After adjusting for confounders, the total effect of a social network on core members' individual performance (β = 0.078, 95% CI: 0.052-0.103) consisted of a direct effect (β = 0.059, 95% CI: 0.030-0.087) as well as an indirect effect mediated through social support (β = 0.019, 95% CI: 0.006-0.033). In addition, self-efficacy was identified as a moderating factor in the relationship between a social network and individual performance, with higher levels of self-efficacy diminishing the influence of a social network on performance outcomes. An extensive social network can enhance social support for core members of SOs caring for the elderly, thereby improving individual performance. Concurrently, targeted interventions should be developed to draw on self-efficacy to activate social network resources and to have a synergistic effect on individual performance.

Keywords: social capital, work performance, care services

1. Introduction

As aging of the global population intensifies, social organizations (SOs) caring for the elderly, as a form of non-governmental organization providing nursing and health care services for older people, have become crucial in addressing the shortcomings of governmental and private care providers (*1,2*). In China, by the end of 2023, there were 387,000 institutions and facilities caring for older adults, with 8.23 million beds available (*3*). This phenomenon is particularly evident in Shanghai, China. As one of China's megacities, Shanghai had a population of 5.6805 million individuals age 60 and older by the end of 2023, representing 37.4% of the total population. The city is home to 700 elderly care institutions, with a combined total of 166,900 beds available (*4*). These organizations provide specialized long-term care services for specific elderly demographics, such as those who are disabled or partially disabled. Additionally, they integrate medical and rehabilitation resources to advance the integration of medical and elderly care, thereby effectively enhancing standards to ensure the health of the local elderly population. In these organizations, the core members, including the organization's legal representative, director, and administrative personnel, bear responsibility for overall management, policy

formulation, financial oversight, and human resource management. Previous research has indicated that these core members influence the care standards and conditions in SOs caring for the elderly through strategic institutional design and team motivation. This helps to enhance the quality of organizational services and the satisfaction levels of older individuals within the organization. Clearly, these core members represent a critical group that deserves attention.

As professionalization within SOs caring for the elderly progresses, the significance of the individual performance of core members has garnered attention from numerous scholars. Individual performance is characterized by the contributions of and influence exerted by these core members, grounded in their professional responsibilities and manifested through their expertise, skills, and collaborative behaviors (*5,6*). Within the context of providing care for older adults, the individual performance of core members plays a pivotal role in optimizing the process of providing care, maintaining team stability, and enhancing the institution's ability to mitigate risks. For older individuals residing in SOs caring for the elderly, ineffective management by core members can lead to a lack of activities, reduce the social participation of older people, and exacerbate adverse health outcomes such as loneliness and depressive symptoms (*7,8*). Previous research has examined individual performance to explore the factors influencing the effectiveness of core members across diverse organizational contexts, and it has yielded mixed findings. A non-interventional study conducted in Poland and Bhutan identified several determinants impacting the performance of business executives, such as managerial vision, evocativeness, membership, and organizational commitment (*9*). Data from 228 administrators in the Yangtze River Delta region of China found that role stress was significantly correlated with managers' innovative performance, and this effect was more pronounced among female managers (*10*). However, the determinants affecting the individual performance of core members within SOs caring for the elderly have yet to be adequately understood.

Considering the significant role that the individual performance of core members plays in improving the operational efficiency of organizations and enhancing the quality of life for older residents, individual performance needs to be comprehensively assessed. This includes exploring influencing factors and their pathways of action, as well as the robust statistical validation of these relationships.

1.1. Conceptual framework

Initially introduced by Bourdieu (*11*), social capital theory highlights the interrelationship between social networks, resource acquisition, and social status. Coleman (*12*) further developed the concept by defining social capital as a public or collective resource that aids individuals or groups in achieving their objectives. At the micro level, research on social capital primarily examines the relational characteristics of individual actors and the influence of their social status on the social capital they can access. At the macro level, research focuses on the structural characteristics of the social networks in which actors are embedded and how interactions and constraints within these networks affect individuals' capacity to access social resources (*13*).

Social capital theory is widely acknowledged as an appropriate theoretical framework for analyzing work performance and has consequently been utilized in numerous related studies (*14-16*). Prior research has demonstrated that variations in social capital can predict self-reported work performance, work engagement, and mental health status among general practitioners at both the individual and group level (*17*). Taken together, findings from social capital research across diverse fields (*18,19*) have revealed that network stressors, such as inadequate structural dimensions (*e.g.*, sparse networks and singular connections), deficient relational dimensions (*e.g.*, low trust and insufficient reciprocity), and lacking cognitive dimensions (*e.g.*, inconsistent goals and information asymmetry), undermine core members' ability to access essential resources and information. Conversely, robust social networks can augment core members' perceived control and organizational commitment, thereby enhancing their work performance through the mechanisms of sharing knowledge and mutual resource support. The current study examines SOs caring for the elderly as a contextual framework for cultivating social capital, with a particular emphasis on social capital at the micro level. This study aims to analyze the impact of the quality of core members' social networks on their work performance.

1.2. Research hypotheses

As mentioned above, a social network represents a crucial component of social capital at the micro level, embodying the relatively stable relational systems established among individuals within society through interactions such as friendships, academic associations, and business partnerships (*15*). The operational characteristics and environments of SOs caring for the elderly necessitate collaboration with entities such as health departments, hospitals, and community organizations to foster synergistic development. This feature leads to opportunities for employees within the organization to establish external connections and enhance their social networks. Empirical evidence indicates that an employee's social network within an organization has a positive influence on their job performance, as individuals with a robust social network can access and draw on additional resources, thereby enhancing their workplace competitiveness (*6*).

Conversely, inadequate social networks can result in a deficiency of essential resources for individuals and also hinder effective communication and collaboration with team members, ultimately diminishing work performance (*20*). Consequently, the following **Hypothesis 1 (H1)** is proposed: A social network positively predicts individual performance.

The social network is posited as a precursor to social support, as it is through social support that individuals access resources such as spiritual, material, and intellectual assets derived from social relationships. Prior research has demonstrated that the breadth and depth of an individual's social network within an organization, encompassing both the quantity and quality of interpersonal relationships, are positively correlated with the levels of emotional, informational, and instrumental support received. This multifaceted social support serves to mitigate work-related stress, enhance the efficiency of resource acquisition, and augment employee competence, thereby substantially enhancing work performance. Therefore, we propose **Hypothesis 2 (H2)**: Social support mediates the relationship between a social network and individual performance.

As a mechanism of human agency, self-efficacy pertains to an individual's confidence in their ability to engage in a specific behavior or achieve a specific goal within a particular domain (*21*), *i.e.*, "I can do it." According to the halo effect in psychology, such self-efficacy facilitates the garnering of trust from others and substantially improves an individual's persuasiveness (*22*). SOs caring for the elderly, characterized by the diverse needs of their care recipients, necessitate periodic innovation in service models by core members of the organization. Concurrently, the intricate interpersonal interactions among core members, older adults, their families, and caregivers invariably contribute to work-related stress and threaten the core members' confidence in their work. Numerous studies have demonstrated that self-efficacy positively influences job performance (*22, 23*). Individuals with a high level of self-efficacy display an enhanced capacity to swiftly adapt to novel demands and sustain consistent job performance within dynamic work settings, such as those characterized by technological advances or organizational changes. Moreover, specific characteristics linked to self-efficacy in core members, such as the capacity to inspire and mobilize, frequently spread throughout the team *via* the organizational leader's self-assurance and positive disposition. These enhance the overall team morale and predispose core members to adeptly lead their teams toward attaining desired objectives.

Resources, information, and knowledge acquired through a social network enable core members to swiftly access essential information, such as market dynamics and technological trends, thereby facilitating more efficient responses to complex issues (*24*). On the one hand, a strong social network can provide emotional encouragement and material help to core members when they face challenges, thus increasing their confidence in their abilities (*25*). On the other hand, by coming into contact with high performers in a social network, core members can indirectly learn problem-solving methods and attitudes, which enhances their ability to access resources (*26*). In addition, recognition from peers within the social network allows core members to attribute their successes to their competencies, reinforcing their self-efficacy. Social networks contribute to developing core members' confidence in their abilities by providing both resource stability and psychological support, which in turn motivates them to tackle challenges and achieve superior performance levels.

Therefore, we propose **Hypothesis 3 (H3)**: Self-efficacy has a moderating effect between a social network and individual performance; **Hypothesis 4 (H4)**: Self-efficacy has a moderating effect between a social network and social support; **Hypothesis 5 (H5)**: Self-efficacy has a moderating effect between social support and individual performance.

In summary, this study develops a moderating mediator model to examine the individual performance of core members within SOs caring for the elderly. It explores the association between the size of core members' social networks and their individual performance, it examines the mediating influence of social support within this relationship, and it validates the moderating effect of self-efficacy. The hypothesized model is shown in Supplementary Figure S1 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=271*).

2. Materials and Methods

2.1. Participants

This study used multi-stage stratified sampling to collect data. Between June and September 2023, core members at SOs caring for the elderly in Shanghai, China, were selected to serve as participants. To ensure the sampling area was representative, Xuhui District, Jing'an District, and Pudong New District were chosen as survey locations based on their geographic positioning and levels of economic development. From each district, a minimum of 20 SOs caring for the elderly were selected according to the development level of SOs within these areas. For each organization, 3 to 5 core members were selected to participate in the survey. The inclusion criteria for participants were: (1) core members such as founders, leaders, legal representatives, and other managers of the organizations and (2) individuals who voluntarily agreed to participate in this study. The exclusion criteria were: (1) individuals who could not provide full responses to the questionnaire and (2) individuals who had been part of the organization for less than one year.

Data were collected by trained and experienced

investigators. Under the guidance of the civil affairs department staff, these investigators visited SOs caring for the elderly to conduct face-to-face interviews with the participants. Once data were collected, the researchers meticulously reviewed and screened the responses to exclude those of substandard quality, specifically: (1) questionnaires completed in less than two minutes, (2) those exhibiting internal logical inconsistencies, and (3) those with incomplete responses. The study surveyed a total of 69 SOs caring for the elderly; as a result, a sample of 213 qualified core members was statistically analyzed.

2.2. Measurement

2.2.1. A social network

The Social Network Scale used in this study is an adaptation of the World Bank's Social Capital Assessment Tool (*27*), which is primarily designed to quantify the number of external social connections of respondents. Specifically, for core members of SOs caring for the elderly, we assessed the number of participants' acquaintances across several domains: civil affairs departments, health commissions, healthcare administrations, neighborhood councils, other government departments, federations of SOs, and other SOs caring for the elderly, encompassing a total of seven categories. For each category, a five-point Likert scale (1-5) was utilized to classify the number of acquaintances of core members (0, 1-5, 6-10, 11-15, and 16 or more), yielding a cumulative score ranging from 7 to 35. A higher score indicates a greater level of social networking. In our study, the Social Network Scale demonstrated a Cronbach's alpha of 0.912, indicating a high level of internal consistency. This scale has undergone extensive validation in prior research conducted across various regions (*6,14,28*).

2.2.2. Social support

The Social Support Scale is a five-item self-report instrument designed to assess the extent of financial, material, emotional, technical, and informational support that individuals receive from friends and colleagues (*27*). Answers are scored using a Likert scale from 1 (strongly disagree) to 5 (strongly agree), and the sum of all item scores constitutes the total score on the Social Support Scale, ranging from 5 to 25 points. Greater social support is represented by a higher score. The scale demonstrated good internal consistency in the current sample, with a Cronbach's alpha of 0.848.

2.2.3. Self-efficacy

The Self-efficacy Scale, devised by Thomas *et al*. (*23*), is widely used to assess perceived personal competence. In the current study, this scale was used to evaluate the self-efficacy of core members at SOs caring for the elderly, focusing on innovation self-efficacy (items 1-3), persuasion self-efficacy (items 4-8), and adaptability self-efficacy (items 9-13). The items were answered by participants using a 5-point Likert scale, where 1 meant strongly disagree and 5 meant strongly agree. The overall self-efficacy score was calculated by summing the scores of all items, with higher scores indicating greater self-efficacy. In this study, the Self-efficacy Scale had a Cronbach's alpha of 0.923.

2.2.4. Individual performance

Based on prior measurement tools for assessing employee performance (*29,30*) and integrating the specific work characteristics of SOs caring for the elderly, this study developed a questionnaire aimed at evaluating the individual performance of core members within these organizations. The questionnaire's development involved multiple rounds of expert consultation to ensure its scientific rigor and practical applicability. The instrument primarily assesses the external influence of core members involved in managing SOs caring for the elderly. It includes items such as: "Have you personally received any awards related to providing care for older adults?" "Has the team you lead received any recognition related to providing care for older adults?" "Have you or your team been featured in the media for the care you provided?" "Are you a member of an association in the area of caring for older adults?" and "Have you participated in drafting or soliciting specifications for providing care for older adults?" Responses are recorded as "No (1 point)" or "Yes (2 points)," and the total score is derived by summing the points across the five items.

2.2.5. Covariates

This study considered various factors that could influence the outcomes, including demographic and occupational variables. Specifically, data were collected on participants' sex (male or female), age group ($\leq$ 40 years, 41-49 years, $\geq$ 50 years), level of education (junior high school or lower, high school, and college or higher), and marital status (married or other). Participants' professional status was classified based on their title, which was categorized as "no" or "yes (junior or above)." Additionally, information was gathered regarding whether participants had received management-related training in the preceding year and the time spent working in the area of providing care for older adults.

2.3. Statistical analysis

Continuous variables were expressed as the mean $\pm$ standard deviation (M $\pm$ SD), while categorical variables were represented by frequency and percentage.

Differences between variables were evaluated using the *t*-test and ANOVA, while Pearson's correlation analysis was used to explore the connections between a social network, social support, self-efficacy, and individual performance. The mediating role of social support in the relationship between a social network and individual performance and the moderating role of self-efficacy were initially assessed using hierarchical stepwise regression. Additionally, data were mean-centered for the calculation of interaction terms. Mediation and moderation models were evaluated using Model 4 and Model 59 from PROCESS version 4.1. The mediation effect was analyzed using the bootstrap method, while the moderation effect was assessed using the simple slope method (*31*).

To evaluate the robustness of the results of linear regression analysis, we performed binary logistic regression analyses to validate the associations among a social network, social support, self-efficacy, and individual performance. Initially, the original continuous independent and dependent variables were transformed into categorical variables. Based on the data distribution, the mean value was utilized to classify the data into "low level (0)" and "high level (1)" categories for a social network, social support, self-efficacy, and individual performance. Additionally, potential confounding effects of covariates were controlled for in the robustness analyses. All statistical tests were performed using SPSS version 23.0, and a P-value<0.05 was deemed statistically significant.

3. Results

3.1. Demographic characteristics

As shown in Table 1, 51 (23.9%) of the core members within the SOs caring for the elderly were male, while 162 (76.1%) were female. Over one-third (35.2%) of the respondents were between 40 and 49 years of age. A substantial proportion of the core members possessed a college degree or higher (81.2%) and were married (86.4%). More than half of the participants held a job title (55.9%) and had undergone management training within the past year (60.6%). In addition, a significant proportion of individuals (57.7%) had been employed in occupations related to caring for older adults for over six years.

Univariate analysis revealed significant differences in social support scores solely related to the age of the respondents. Moreover, the individual performance scores of core members differed significantly based on how long they were engaged in work related to caring for older adults.

3.2. Correlation analysis

Supplementary Table S1 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=271*) presents the findings of Pearson's correlation analysis, indicating that a more extensive social network is significantly linked to better scores for social support ($r = 0.497$, $P < 0.001$), individual performance ($r = 0.431$, $P < 0.001$), and self-efficacy ($r = 0.376$, $P < 0.001$) among participants. Moreover, social support positively correlated with individual performance and self-efficacy. Individual performance positively correlated with self-efficacy.

3.3. Mediating role of social support in a social network and individual performance

In this study, a hierarchical stepwise regression analysis was performed on the control variables that differed significantly in the univariate analysis of variance. Findings indicated that core members of SOs caring for the elderly with a more extensive social network were more likely to receive higher levels of social support ($\beta = 0.508$, $P < 0.001$). Moreover, in stratified stepwise regression analysis where individual performance was the explanatory variable, the level of one's social network was found to positively predict individual performance ($\beta = 0.381$, $P < 0.001$), thereby substantiating Hypothesis 1. Additionally, participants who provided care to older adults for a longer period had a higher likelihood of achieving elevated individual performance levels. As a result of incorporating social support into the regression model, the R^2 value went up from 0.251 to 0.277, and the standardized regression coefficient for a social network decreased from 0.381 to 0.288. This suggests that social support mediates the relationship between a social network and individual performance (Table 2A).

The mediating effect was evaluated using the bootstrap method, specifically employing Model 4 in PROCESS version 4.1, with a resampling rate of 5,000 iterations. Results indicated that social networks had a total effect of 0.078 on individual performance, with a 95% confidence interval (CI) between 0.052 and 0.103. The indirect mediating effect *via* social support was calculated to be 0.019, with a 95% CI of 0.006 to 0.033. These findings indicate that social support is a significant mediator in the relationship between the social network of core members of SOs caring for the elderly and individual performance, accounting for 24.44% of the variance in the total effect. Consequently, Hypothesis 2 was confirmed, as detailed in Table 3.

This study performed a robustness analysis using binary logistic regression, as shown in Supplementary Table S2 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=271*), which presents results comparable to those obtained from hierarchical stepwise regression. In the fully adjusted model, respondents with a more extensive social network (OR = 2.206; 95% CI: 0.999-4.872), social support (OR = 3.076; 95% CI: 1.439-6.773), and self-efficacy (OR = 2.515; 95% CI: 0.705-3.257) were more likely to exhibit high

Table 1. Demographic characteristics and scores ($N = 213$)

Variables	N (%)	Social network		Social support		Individual performance		Self-efficacy	
		M ± SD	*t/F*	M ± SD	*t/F*	M ± SD	*t/F*	M ± SD	*t/F*
Sex									
Male	51 (23.9)	16.02 ± 7.77	1.535	21.47 ± 3.35	-0.125	6.76 ± 1.39	0.225	58.57 ± 6.16	2.738*
Female	162 (76.1)	14.19 ± 6.17		21.54 ± 3.29		6.72 ± 1.34		55.40 ± 7.52	
Age (years)									
≤ 39	69 (32.4)	13.65 ± 5.71	1.151	22.65 ± 2.68	7.041*	6.43 ± 1.21	2.449	55.93 ± 8.01	0.343
40-49	75 (35.2)	15.24 ± 7.62		21.28 ± 3.27		6.87 ± 1.34		55.81 ± 7.30	
50-59	69 (32.4)	14.94 ± 6.26		20.65 ± 3.60		6.87 ± 1.45		56.75 ± 6.70	
Level of education									
Junior high school or lower	14 (6.6)	10.71 ± 3.56	3.157*	21.86 ± 2.93	1.454	6.64 ± 1.22	0.324	52.64 ± 7.69	1.835
High school	26 (12.2)	13.73 ± 6.50		20.50 ± 4.08		6.92 ± 1.60		57.00 ± 8.04	
College or higher	173 (81.2)	15.08 ± 6.73		21.64 ± 3.18		6.71 ± 1.32		56.31 ± 7.15	
Professional title									
Yes	119 (55.9)	15.34 ± 7.04	1.176	21.61 ± 3.24	0.459	6.85 ± 1.33	1.481	56.76 ± 6.71	1.350
No	94 (44.1)	13.73 ± 5.95		21.40 ± 3.37		6.57 ± 1.36		55.39 ± 8.01	
Marital status									
Married	184 (86.4)	15.03 ± 6.66	2.264*	21.46 ± 3.37	-0.659	6.81 ± 1.34	2.263*	56.27 ± 7.33	0.558
Other	29 (13.6)	12.07 ± 5.81		21.90 ± 2.83		6.21 ± 1.26		55.45 ± 7.43	
Management training									
Yes	129 (60.6)	16.32 ± 6.96	5.180**	21.60 ± 3.51	0.415	6.99 ± 1.38	3.656**	57.43 ± 6.81	3.206*
No	84 (39.4)	12.03 ± 5.08		21.40 ± 2.95		6.32 ± 1.19		54.20 ± 7.70	
Time spent working (years)									
≤ 5	90 (42.3)	13.96 ± 6.37	0.824	21.77 ± 3.37	0.437	6.37 ± 1.24	8.624**	56.18 ± 7.34	0.608
6-10	64 (30.0)	15.02 ± 6.25		21.38 ± 2.97		6.73 ± 1.34		55.43 ± 7.48	
≥ 11	59 (27.7)	15.24 ± 7.36		21.31 ± 3.54		7.27 ± 1.35		56.90 ± 7.19	

*$P < 0.05$, **$P < 0.001$.

Table 2. Results of hierarchical stepwise regression

A	Social support				Individual performance		
	Model 1	Model 2			Model 1	Model 2	Model 3
Age	-0.099	-0.139**		Marital status	-0.077	-0.034	-0.022
Social network		0.508**		Management training	-0.198*	-0.083	-0.085
				Time spent working	0.224*	0.221**	0.226**
				Social network		0.381**	0.288**
				Social support			0.189*
F	2.103	38.130**		*F*	9.659**	17.390**	15.897**
R^2	0.010	0.266		R^2	0.122	0.251	0.277
ΔR^2	0.005	0.259		ΔR^2	0.109	0.236	0.260
B	Social support				Individual performance		
	Model 1	Model 2	Model 3		Model 1	Model 2	Model 3
Age	-0.099	-0.143*	-0.143**	Marital status	-0.077	-0.025	-0.020
Social network		0.420**	0.436**	Management training	-0.198*	-0.075	-0.059
Self-efficacy		0.236**	0.227**	Time spent working	0.224*	0.226**	0.229*
SN*SE			-0.051	Social network		0.270**	0.274**
				Social support		0.166*	0.169**
				Self-efficacy		0.187*	0.184*
				SN*SE			-0.133*
				SS*SE			-0.034
F	2.103	31.910**	24.078**		9.659**	13.583**	10.816**
R^2	0.010	0.314	0.316		0.122	0.283	0.298
ΔR^2	0.005	0.304	0.303		0.109	0.263	0.270

*P < 0.05, **P < 0.001, A: Results of mediation analysis by hierarchical stepwise regression; B: Results of moderation analysis by hierarchical stepwise regression. SN: social network, SE: self-efficacy, SS: social support.

levels of individual performance. Moreover, respondents who were employed in providing care for older adults for 11 years or longer had higher levels of individual performance than core members employed in providing care for older adults for five years or less.

3.4. Moderating effects of self-efficacy on a social network and individual performance

Table 2B summarizes the results from the analysis of moderating effects. The interaction term between a social network and self-efficacy did not significantly predict social support (β = -0.051, P = 0.399). Similarly, the interaction term between social support and self-efficacy did not significantly predict individual performance (β = -0.034, P = 0.689), leading to the rejection of Hypotheses 4 and 5. Conversely, the interaction term between a social network and self-efficacy emerged as a significant negative predictor of individual performance (β = -0.133, P = 0.023), indicating that self-efficacy is a negative moderating factor between a social network and individual performance.

Building on the aforementioned results, the moderating effect was further examined in Model 59 in PROCESS version 4.1 using the bootstrap method. As shown in Supplementary Table S3 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=271*), the 95% CIs for the moderating effects across all three levels of the pathway linking a social network to individual performance do not include zero.

Table 3. Results of mediating effect analysis with the Bootstrap method

	Effect size	Bootstrap SE	95% CI	*P* value
Total effect	0.078	0.013	0.052, 0.103	< 0.001
Direct effect	0.059	0.014	0.030, 0.087	< 0.001
Indirect effect	0.019	0.007	0.006, 0.033	< 0.001

This indicates that self-efficacy serves as a moderating factor between a social network and individual performance. The simple slope analysis depicted in Figure 1 reveals that core members with a higher level of self-efficacy who have a more extensive social network will experience a decrease in their individual performance relative to participants with a lower level of self-efficacy, aligning with Hypothesis 3. The final moderated mediation model is shown in Figure 2. The discriminant validity of the variables was further tested by performing confirmatory factor analyses (CFAs) on the observed data. The measurement model fit the data acceptably (χ^2/df = 2.71, RMSEA = 0.078, SRMR = 0.074, CFI = 0.923, TLI = 0.916).

4. Discussion

Despite the mounting empirical evidence supporting the impact of a social network on individual performance within organizations, the mediating and moderating pathways are still not yet adequately understood. In this study, we developed a moderating mediation model

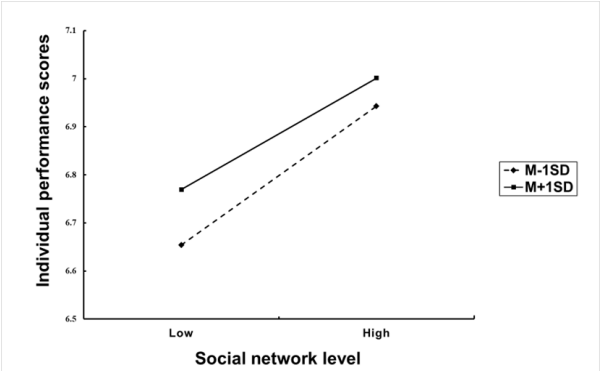

Figure 1. The moderating role of self-efficacy between a social network and individual performance. This figure illustrates how self-efficacy moderates the relationship between a social network (divided into low and high levels) and individual performance. The solid line (M+1SD) represents the scenario where self-efficacy is one standard deviation above the mean, and the dotted line (M-1SD) represents self-efficacy one standard deviation below the mean.

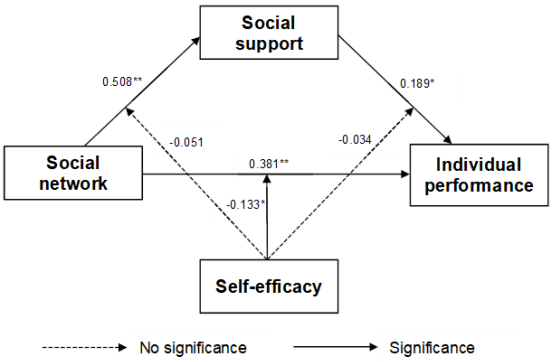

Figure 2. The moderated mediation model. A social network affects individual performance directly (path coefficient = 0.381) and indirectly *via* social support (social network → social support: 0.508; social support → individual performance: 0.189). Self-efficacy moderates paths (social network → individual performance: -0.133). Dotted lines indicate non-significant moderation effects, **P* < 0.05, ***P* < 0.001.

focusing on the performance of core members of SOs caring for the elderly. Findings indicated that social networks have a significant positive relationship with the individual performance of core members of SOs caring for the elderly, with social support serving as a partial mediator in this relationship. Moreover, self-efficacy moderates the relationship between a social network and individual performance. Specifically, higher levels of self-efficacy among core members attenuate the positive effect of a social network on individual performance.

4.1. Differences in individual performance

This study assessed the levels of individual performance of these core members by evaluating external factors influencing those involved in the management of caring for older adults. The average score for respondents' individual performance was 6.72 ± 1.35, indicating a low to medium level of performance. A prior study found that the leadership performance of managers in US nursing homes, a key metric for job performance, had a mean score of 3.62 on a 5-point scale, with high-performing managers associated with significantly lower employee turnover rates and partially better nursing quality outcomes (*32*). Existing studies have predominantly focused on employee performance and factors influencing it in medical facilities, educational settings, and companies (*33-37*). Performance assessment standards for core members of SOs caring for the elderly in China are typically established by the organizations or their respective parent departments (*6*). These standards lack uniformity across different organizations, and performance outcomes need to be more closely integrated with the salaries and positions of core members. The findings of this study offer additional empirical evidence regarding the levels of job performance within this demographic.

In univariate analysis, married participants had higher individual performance scores. This finding aligns with previous research, such as a cross-sectional study conducted in Anhui Province, China, which revealed that married core members at nursing facilities were likelier to receive awards or recognition for their work (*6*). From a mechanistic perspective, emotional support from spouses or families of married individuals contributes to sustained work engagement and enhanced performance levels. Moreover, core members with prior managerial training also had higher individual performance scores. The knowledge acquired during training, encompassing organizational strategic planning, human resource management, and financial management, proved advantageous in enabling core members to implement standardized processes and enhance work efficiency.

Moreover, the evidence indicates that experience in providing care for older adults positively influences the individual performance of core members, as demonstrated in multiple rounds of analysis. A previous meta-analysis corroborates these findings, revealing that triage by a senior physician in hospitals enhances hospital performance metrics and patient satisfaction (*38*). A study involving 112 non-profit providers of care for the elderly in Japan found that managers with over a decade of management experience were more inclined to implement stringent budget control strategies to enhance their organizations' financial outcomes (β=0.27, P<0.01) (*39*). Within SOs caring for the elderly, core members with extensive experience can attract social resources to the organization, facilitating stable partnerships with volunteer organizations and hospitals (*e.g.*, regular volunteer activities) and broadening the scope of services offered (*40,41*).

4.2. Mediating effects of social support

This study discovered that the size of a social network among core members of SOs caring for the elderly positively predicts their individual performance. Although previous research has focused on different populations, it has extensively documented the influence of a social network on the job performance of organizational core members. Data collected from a survey of 340 female leaders employed in a multinational organization highlighted that managerial performance is affected not only by leaders' personal traits and leadership styles but also by the configuration of their social network (*42*). Pilar *et al*. (*43*) performed a social network analysis and found that extensive external connections significantly enhanced physicians' performance at both the individual and team level. This improvement was attributed to seeking advice through social networks outside the workplace. Focusing on charity shop managers in the UK, a comprehensive study revealed that interacting with colleagues and volunteers can alleviate managers' dissatisfaction with their status and have a positive effect on job satisfaction and performance (*44*). Similarly, a study conducted in China indicated that core members of an organization interact with external entities through the sharing of information, collaboration, and decision-making, highlighting the critical role of building a social network in enhancing performance (*45*). Establishing connections with government departments, medical facilities, and other SOs caring for the elderly is essential for the effective operation of SOs caring for the elderly. Suppose that core members create a diverse social network both within and outside the organization, such as strong ties with medical teams, family members, and industry organizations. In that case, there will be significant improvements in performance metrics, including service quality, operational cost control, and employee stability (*28,46*).

Social support serves as a mediating variable in the relationship between a social network and individual performance. Within SOs caring for the elderly, care recipients range widely in age and have varying levels of functioning, resulting in varied care needs and differing levels of organizational competence (*16,47*). The managerial roles of core members are marked by high stress and emotional exhaustion, necessitating emotional, instrumental, and cognitive support through a social network to mitigate burnout and enhance managerial resilience. In the current study, the measurement tool for social support incorporates multiple sources, including financial, material, emotional, technological, and informational support. Specifically, an extensive social network offers core members opportunities for exchanges with peers (*e.g.*, industry seminars), professional guidance from superiors (*e.g.*, training on policy interpretation), and the building of trust within the team. These forms of social support can be effectively translated into enhanced work effectiveness (*14*). For instance, when core members encounter conflicts with the families of care recipients, a legal advisor's social support can assist the member in reaching a resolution (*48*). In situations involving issues with staff motivation, exchanging experiences among peers can lead to innovative management strategies for core members.

4.3. Moderating effect of self-efficacy

The current findings corroborated the moderating role of self-efficacy in the relationship between a social network and individual performance, indicating that elevated levels of self-efficacy attenuate the positive impact of a social network on individual performance. Contrary to our findings, a study in southern China found that managers with higher self-efficacy experienced a stronger positive impact on performance from aligning their work passions than those with lower self-efficacy (*49*). Similarly, findings from South Korea identified self-efficacy as a significant predictor of nursing performance, accounting for 21.9% of the variance (*50*). Prior research has demonstrated that some occupational aspects, including job satisfaction, job knowledge, and personality traits, enhance performance by augmenting self-efficacy (*51-53*). This variance can be attributed to two primary factors. On the one hand, within the framework of social cognitive theory, self-efficacy reflects a person's belief in their own capabilities. Individuals with high self-efficacy are inclined to depend on internal resources, such as personal experience and decision-making skills, rather than external social network resources for problem-solving. For example, core members with high self-efficacy may rely solely on personal experience when devising management programs, potentially failing to engage with healthcare facilities or other SOs caring for the elderly (*54*). In resolving employee conflicts, an over-reliance on personal authority, rather than employing mediation strategies through peer networks, may intensify team conflicts (*55*).

On the other hand, when individuals believe their abilities are adequate to address challenges, the perceived value of a social network as an "alternative resource" is diminished. In managing SOs caring for the elderly, core members with high self-efficacy may independently devise reforms to enhance care processes. However, they often face obstacles in implementation due to insufficient feedback from caregivers (*56*). Additionally, core members who excessively depend on their personal experience may overlook innovative case studies from industry networks, potentially resulting in a rigid management model (*57*). An important point to emphasize is that this negative regulation does not undermine the positive role of self-efficacy but rather highlights its dynamic interaction with social networks. In future practice, SOs caring for the elderly need to bolster core members' self-efficacy, enhance their understanding of the strategic importance of social network resources,

and develop incentive mechanisms that encourage core members to convert their self-confidence into a catalyst for activating network resources.

4.4. Practical contributions

In addition to the aforementioned theoretical contributions, this study offers practical implications for improving the individual performance of core members of SOs caring for the elderly and for enhancing the quality of care. Firstly, multi-level social networking channels should be established and maintained. SOs caring for the elderly should regularly facilitate cross-departmental and cross-level communication activities, such as "management salons" to dismantle information silos within the organization. Concurrently, a dedicated online collaboration and knowledge-sharing platform should be created, enabling core members to initiate discussions, disseminate information, and seek assistance at any time. Secondly, the organizational provision of social support needs to be enhanced. SOs caring for the elderly should develop a comprehensive, multi-dimensional support framework. This framework should include "senior mentors" who possess an in-depth understanding of the organization's operations, as well as "cross-border consultants" sourced from external entities. Beyond offering guidance on professional skills, these mentors and consultants should also provide emotional and cognitive support. Finally, differentiated attention is paid to the boundary effects of self-efficacy. On the one hand, SOs caring for the elderly should facilitate the empowerment of core members with low self-efficacy by encouraging them to actively seek support and draw on network resources. This can be achieved through targeted programs such as "Social Capital Workshops" and "Interpersonal Communication Training," aimed at optimizing their performance. On the other hand, core members with high self-efficacy should be given challenging tasks and opportunities for change. Organizations can assign them more independent and complex projects, such as cross-departmental reforms and process optimizations, to stimulate their intrinsic motivation.

4.5. Limitations and future prospects

This study had several limitations. One such limitation was that the cross-sectional design used in this study precluded our ability to explicitly investigate the causal mechanisms linking a social network and individual performance. Future studies should consider utilizing a longitudinal design to rigorously test the proposed moderated mediation model. Another limitation is that this study posited and examined the moderating role of self-efficacy in the relationship between a social network and individual performance. However, the literature suggests that some career-related aspects, such as job satisfaction, job knowledge, and personality traits, can augment performance levels by enhancing self-efficacy. Future research should explore the mediating role of self-efficacy in the association between individual performance by core members and its impacts across other domains. The data analyzed in this study were obtained from SOs caring for the elderly in Shanghai. Future studies should compare situations across regions and cities with different levels of economic development to examine potential differences in the mediating and moderating effects identified in this study across regional strata.

In addition, this study posits that self-efficacy will attenuate the positive impact of social networks on the individual performance of core members of SOs caring for the elderly. However, a point that needs to be acknowledged is self-efficacy's role in facilitating job performance as documented in the literature. Consequently, the generalizability and replicability of these findings warrant further investigation using samples from diverse cultural contexts.

5. Conclusion

This study has elucidated how social networks influence individual performance, emphasizing the mediating role of social support, particularly among core members of SOs caring for the elderly. Notably, heightened self-efficacy among these core members mitigates the positive impact of a social network on individual performance. Theoretically, this research adds to the literature by substantiating the mediating effect of social support and the moderating effect of self-efficacy, thereby enhancing our understanding of the relationship between the social networks and individual performance of core members of SOs caring for the elderly. Practically, these findings offer crucial insights for devising and implementing comprehensive intervention strategies aimed at enhancing the quality of care and the performance of core membership teams within these organizations.

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Ethics approval and consent to participate: All participants gave written informed consent after

thoroughly understanding the information provided. Additionally, all participants demonstrated the medical and ethical capacity to provide consent. The study protocol was approved and ethical clearance was granted by the Biomedical Ethics Committee at Anhui Medical University (No. 20180181). In addition, all methods used in this study are in accordance with the Declaration of Helsinki.

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Original Article

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Integrative methylation and transcriptomic analysis reveals key genes linking cellular senescence and metabolic reprogramming in colorectal liver metastasis

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SUMMARY: Colorectal liver metastasis (CRLM) remains lethal, and the convergence of cellular senescence with metabolic reprogramming *via* epigenomic rewiring is poorly understood. We integrated genome-wide DNA methylation and RNA-seq data from 10 paired primary tumors and liver metastases (GSE213402). After calling differentially methylated genes (3,399 hyper- and 9,519 hypomethylated) and differentially expressed genes (406 DEGs), we intersected them with curated senescence (n = 866) and metabolic reprogramming (n = 948) gene sets, yielding 28 differentially expressed cellular-senescence-related genes (DE-CSRGs) and 24 metabolic-reprogramming-related genes (DE-MRRGs). Machine-learning pipelines (LASSO + SVM-RFE) converged on a five-gene signature: *CXCL1*, *SERPINE1*, *NDRG1*, *SRM* and *GATM*, most of which are hypomethylated and over-expressed in metastases. Gene-set enrichment analysis revealed that these genes are involved in pathways such as oxidative phosphorylation, focal adhesion, complement–coagulation cascades, and PPAR signaling. Immune de-convolution revealed strong positive correlations between signature genes and immunosuppressive subsets (MDSCs, Tregs, type-1 T-helper cells; $p < 0.05$). Elevated IC_{50} values for oxaliplatin and 5-fluorouracil in metastatic samples were positively associated with *NDRG1* and negatively with *SRM*, indicating chemo-resistance modulation. This five-gene epigenetic–transcriptomic hub identifies a molecular signature that warrants prospective validation as a potential biomarker for patient stratification and combination therapy in CRLM.

Keywords: DNA hypomethylation, machine-learning signature, chemoresistance, immunosuppressive microenvironment, polyamine metabolism

1. Introduction

Colorectal liver metastasis (CRLM) remains a major clinical challenge, as colorectal cancer (CRC) is the second leading cause of cancer-related mortality globally, and the liver is the primary site of metastasis. CRLM occurs in 20–50% of patients with Colorectal Cancer (CRC), with a 5-year survival rate of 10–30%, depending on the resectability of metastases (*1*). Current treatment options include surgical resection, systemic chemotherapy, radiation therapy, and ablation therapy; however, only a minority of patients are eligible for curative resection (*2*). Immunotherapy has emerged as a promising therapeutic approach, particularly when combined with local therapies. Nonetheless, prognosis remains poor, underscoring the need for novel biomarkers to improve prognostic accuracy and guide personalized treatment strategies.

Cellular senescence is an irreversible cell cycle arrest triggered by stressors, such as DNA damage and oxidative stress (*3*). It is characterized by enlarged cell size, expression of cell cycle inhibitors (*e.g.*, p16 and p21), and a senescence-associated secretory phenotype (SASP), which includes pro-inflammatory cytokines and extracellular matrix (ECM) remodeling proteins (*4*). Senescence can act as a tumor suppressor by halting the proliferation of damaged cells (*5*); however, SASP can also promote tumor progression by altering the tumor microenvironment (*6*). Metabolic reprogramming, which involves adaptive changes in the cellular metabolism that support rapid proliferation, is a hallmark of cancer cells (*7*). In CRLM, metabolic reprogramming enables cancer cells to adapt to the liver microenvironment, facilitating metastatic colonization (*8,9*). The interplay between senescence and metabolic reprogramming in CRLM is complex, with senescent cells potentially

inducing metabolic changes in neighboring cells, and metabolic alterations reciprocally influencing senescent phenotypes. Identifying biomarkers associated with these processes is crucial for elucidating the pathogenesis of CRLM and may provide novel therapeutic targets.

In this study, we utilized publicly available methylation and transcriptomic data on CRLM to investigate the crucial roles and prognostic value of genes related to cellular senescence and metabolic reprogramming in disease progression, employing bioinformatic techniques. We further investigated potential molecular regulatory mechanisms and drug responses associated with these genes. Our findings provide novel theoretical support and a valuable reference for clinical research and prognostic evaluation in CRLM.

2. Materials and Methods

2.1. Sample collection and RNA-sequencing

Fresh tissue samples, including primary colorectal tumors and matched liver metastases, were collected from patients diagnosed with CRC at HuaShan Hospital between 2014 and 2020. All samples were obtained immediately following surgical resection, snap-frozen in liquid nitrogen, and stored at –80 °C until further processing. All clinical samples were collected after obtaining informed consent and in accordance with a protocol approved by the Ethics Committee of Huashan Hospital, Fudan University (Shanghai, China). All clinical samples were collected from patients after obtaining informed consent in accordance with a protocol approved by the Ethics Committee of Huashan hospital, Fudan University (Shanghai, China).

2.2. Data source

RNA sequencing data and clinical information were obtained from the TCGA database *via* UCSC Xena (*http://xena.ucsc.edu/*). TCGA-COAD and TCGA-READ data were merged to generate a TCGA-CRC dataset, and only samples with available survival information were retained. Ultimately, 607 tumor samples and 51 normal samples from the TCGA were used for prognostic analysis. The transcriptome and DNA methylation data of 10 paired primary tumors and liver metastases from 10 patients with CRC were sourced from the GSE213402 dataset in the GEO database to screen for metastasis-related genes. In total, 866 cellular senescence-related genes (CSRGs) were obtained from the CellAge database (*https://genomics.senescence.=cells/*), and 948 metabolic reprogramming-related genes (MRRGs) were identified from a literature search (*10*).

2.3. Identification of methylated genes involved in metastasis

First, we identified differentially methylated CpGs (DMCs) between liver metastasis and primary tumor groups using thresholds of p-value < 0.05 and $|\Delta\beta| > 0.2$ and investigated the distribution of DMCs across different CpG regions. Next, we mapped CpG sites to their corresponding genes to identify the methylated genes involved in metastasis. Genes were classified into distinct methylation states based on the ratio of hypermethylated to hypomethylated CpGs, using a 1.5-fold threshold. Specifically, genes with a hypermethylated/hypomethylated CpG ratio ≥ 1.5 were defined as hypermethylated, and those with a hypomethylated/hypermethylated ratio ≥1.5 were defined as hypomethylated. To analyze the biological pathways associated with methylated genes, functional enrichment analysis was conducted using the R package "clusterProfiler."

2.4. Identification of differentially methylated CSRGs and MRRGs

We used the DESeq2 package to identify differentially expressed genes (DEGs) between the liver metastasis and primary tumor groups, with a false discovery rate (FDR) $P_{adj} < 0.05$ and $|\log2FC| > 1$. To identify differentially expressed cellular senescence-related genes (DECSRGs) and metabolic reprogramming-related genes (DEMRRGs), we performed an overlap analysis among DEGs, 866 CSRGs, and 948 MRRGs. Differentially methylated cellular senescence-related genes (DM-CSRGs) were identified through intersection analysis of (*i*) hypermethylated genes with downregulated DECSRGs, and (*ii*) hypomethylated genes with upregulated DECSRGs. Similarly, differentially methylated metabolic reprogramming genes (DM-MRRGs) were derived from the intersections of (*i*) hypermethylated genes and downregulated DEMRRGs, and (*ii*) hypomethylated genes and upregulated DEMRRGs. Spearman's correlation analysis was conducted on DM-CSRGs and DM-MRRGs to evaluate their association. Correlated DM-CSRGs and DM-MRRGs were screened based on a threshold of $p < 0.05$ and $|cor| > 0.3$.

2.5. Machine learning

To identify key genes, two machine learning algorithms, support vector machine-recursive feature elimination (SVM-RFE) and least absolute shrinkage and selection operator (LASSO), were employed. Initially, we utilized the 'glmnet' package in R for LASSO analysis, which selects key genes from a set of candidate genes through binomial logistic regression. Subsequently, the SVM package was employed to implement the SVM-RFE model using a 10-fold cross-validation strategy. Key CSRGs and MRRGs were identified by overlapping the feature genes obtained from LASSO and SVM-RFE.

2.6. Characterization of key genes

First, we analyzed the expression levels of key genes in the primary tumors and liver metastases. Subsequently, to investigate the prognostic value of the key genes in CRC, we divided TCGA-CRC samples into high- and low-expression groups based on the median expression levels of the key genes, followed by Kaplan–Meier (KM) survival analysis. Gene set enrichment analysis (GSEA) was performed to investigate the functions of key genes using c2.cp.kegg.v7.5.1.symbols.gmt gene set as the reference. Subcellular localization analysis of key genes was performed using Cell-Ploc 2.0.

2.7. Analysis of immune microenvironment and drug sensitivity

The infiltration of 28 immune cell types, including B cell memory, eosinophil, macrophage, mast cell, monocyte, neutrophil, NK cell, effector memory CD4 T cell, effector memory CD8 T cell, activated CD4 T cell, activated CD8 T cell, T cell follicular helper, T cell gamma delta, T cell regulatory, activated B cell, activated dendritic cell, CD56bright natural killer cell, CD56dim natural killer cell, central memory CD4 T cell, central memory CD8 T cell, immature B cell, immature dendritic cell, MDSC, natural killer T cell, plasmacytoid dendritic cell, T cell helper cell type 1, T cell helper cell type 2, and T cell helper cell type 17 were compared between liver metastasis and primary tumor groups. Additionally, the half-maximal inhibitory concentration (IC_{50}) values of irinotecan (1088), oxaliplatin (1089), and 5-fluorouracil (1073) were calculated using the "oncoPredict" R package to assess the drug sensitivity of patients with primary tumor and liver metastasis.

2.8. Statistical analysis

A regulatory network was constructed using Cytoscape (version 3.9.1). All statistical results were analyzed using R (version 4.4.0). KM curves and log-rank tests were used to visualize and test the survival differences among the different groups. Differences in the continuous variables between the two groups were examined using the Wilcoxon test. A p-value < 0.05, unless otherwise specified, was considered statistically significant.

3. Results

3.1. Analysis of the landscape of methylation in the metastasis of CRC

We performed differential methylation analysis and identified 98,858 DMCs between the liver metastasis and primary tumor groups, including 31,883 hypermethylated and 66,975 hypomethylated sites (Figure 1A). The genomic distribution of these DMCs across the 24 chromosomes revealed that the majority of DMCs were hypomethylated (Figure 1B). Moreover, the proportions of hypomethylation in CpG island core, CpG island shelf, and CpG island shores were 53.7%, 67.5%, and 62.8%, respectively, exceeding the corresponding proportions of hypermethylation (Figure 1C). Based on the CpG sites, 3,399 hypermethylated genes and 9,519 hypomethylated genes were identified. The enrichment results of Gene Ontology showed that genes were associated with biological processes such as embryonic organ development, axonogenesis, synapse organization, cellular components related to synaptic membrane, neuronal cell body, glutamatergic synapse, and molecular functions such as GTPase regulator activity, DNA-binding transcription activator activity, and phospholipid binding (Figure 1D). Kyoto Encyclopedia of Genes and Genomes analysis revealed that these genes were involved in the PI3K-Akt signaling pathway, human papillomavirus infection, MAPK signaling pathway, gastric cancer, breast cancer, and ECM-receptor interaction (Figure 1E).

3.2. Identification of DM-CSRGs and DM-MRRGs

We identified 406 DEGs between the liver metastasis and primary tumor groups, including 250 upregulated and 156 downregulated genes in the primary tumor group (Figure 2A). By overlapping the 866 CSRGs and 948 MRRGs, 28 DECSRGs and 24 DEMRRGs were identified (Figure 2B). Eight DM-CSRGs (*CXCL1*, *NDRG1*, *SERPINE1*, *WSB1, CCL2, EGR2, XAF1*, and *VCAN*) and seven DM-MRRGs *(IL4I1, DPYD, ALOX5, CYP1B1, HNMT, SRM*, and *GATM*) were identified (Figure 2C). Among these, *CXCL1* and *SRM* were hypermethylated, whereas *NDRG1*, *SERPINE1*, *WSB1, CCL2, EGR2, XAF1, VCAN, IL4I1, DPYD, ALOX5, CYP1B1, HNMT,* and *GATM* were hypomethylated. We observed multiple correlations among these genes; *SRM* exhibited a strong positive correlation with *CXCL1* (r = 0.71). *SERPINE1* positively correlated with DPYD (r = 0.60), ALOX5 (r = 0.58), and CYP1B1 (r = 0.64). CCL2 expression was positively associated with the expression of IL4I1 (r = 0.60), DPYD (r = 0.90), ALOX5 (r = 0.58), and CYP1B1 (r = 0.88). VCAN also positively correlated with IL4I1 (r = 0.59), DPYD (r = 0.63), ALOX5 (r = 0.62), and CYP1B1 (r = 0.78). Conversely, a negative correlation was detected between *CXCL1* and HNMT (r = -0.56) (Figure 2D).

In the GSE213402 dataset, the expression levels of *GATM*, *NDRG1*, and *SERPINE1* were elevated, whereas those of *CXCL1* and *SRM* were reduced in the liver metastasis group compared with those in the primary tumor group (Figure 3A). The expression patterns of these genes were consistent with the sequencing data (Figure 3B). Additionally, the expression levels of *CXCL1*, *SERPINE1*, and *SRM* were upregulated, whereas those of *GATM* and *NDRG1* were downregulated in

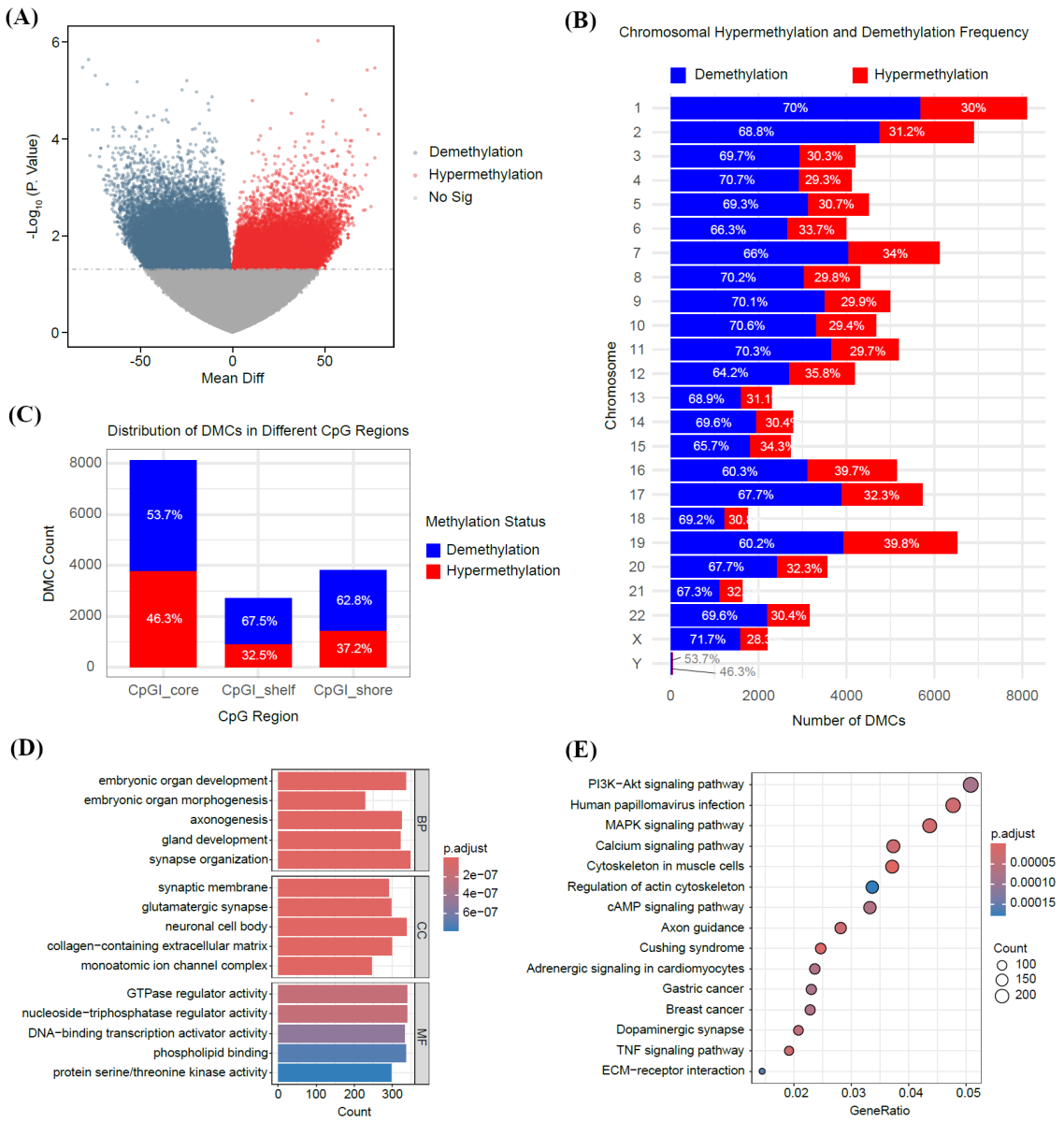

Figure 1. Genome-wide methylation landscape distinguishing colorectal liver metastasis from primary tumors. (A) Volcano plot of 98,858 differentially methylated CpG sites (DMCs; $|\Delta\beta| > 0.2$ and $p < 0.05$) between liver metastasis and primary tumor samples in the GSE213402 cohort. Hypermethylated probes ($n = 31,883$) are shown in red; hypomethylated probes ($n = 66,975$) are shown in blue. **(B)** Bar chart showing the number of hypermethylated (red bars) and hypomethylated (blue bars) DMCs across the 24 human chromosomes. **(C)** Proportional distribution of DMCs within CpG-island sub-regions: CpG-island core, shore and shelf. Hypomethylation predominates in all three compartments (53.7 %, 62.8 % and 67.5 %, respectively). **(D)** Top Gene Ontology (GO) biological-process and molecular-function terms enriched among genes harbouring metastasis-associated DMCs. Bars represent –log10 (adjusted p value); the dashed vertical line indicates adjusted $p = 0.05$. **(E)** KEGG pathway enrichment bubble chart for the same gene set. Bubble size is proportional to the number of genes; colour intensity reflects –log10 (adjusted p value). Key cancer-related pathways (PI3K-Akt, MAPK, ECM-receptor interaction) are highlighted.

the tumor group compared with those in the control group (Figure 3C). The expression trends of *GATM*, *SRM*, *CXCL1*, and *NDRG1* were consistent with our sequencing data (Figure 3D). Furthermore, KM curves were plotted for the five genes, and significant survival differences were observed for *CXCL1* ($p = 0.031$) and *GATM* ($p = 0.038$), indicating their potential as prognostic biomarkers (Figure 3E). We performed GSEA to gain a deeper understanding of the potential mechanisms and found that *NDRG1* and *SERPINE1* were associated with ECM-receptor interaction, focal adhesion, Leishmania infection, lysosome, complement, and coagulation cascades. *GATM* and *SRM* were related to ribosomes, Huntington's disease, Parkinson's disease, and oxidative phosphorylation (OXPHOS), while *CXCL1* was associated with the PPAR signaling pathway (Figure 4A-4E).

3.3. Machine learning identified five key cellular senescence-metabolic genes

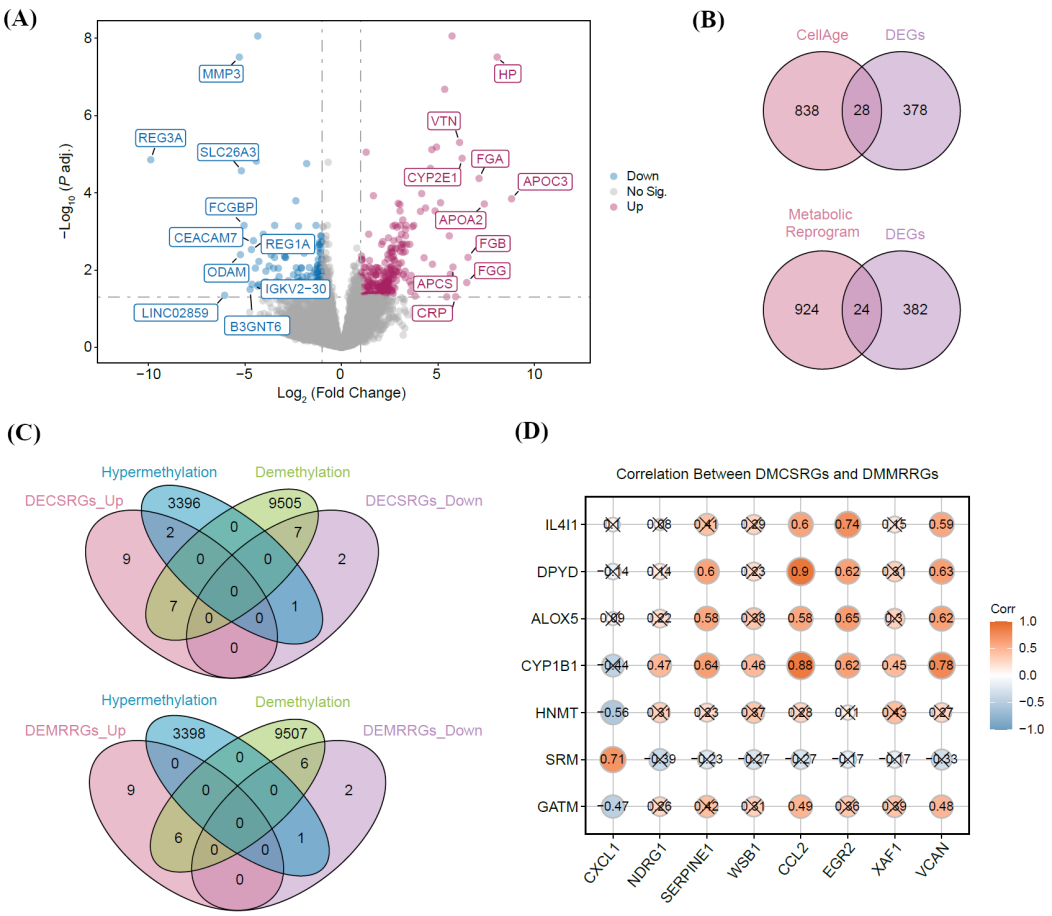

Figure 2. Identification and epigenetic–transcriptional coupling of senescence- and metabolism-related genes in CRLM. (A) Volcano plot of differentially expressed genes (DEGs) between liver metastasis and primary tumour samples (GSE213402). Red, up-regulated genes (n = 250); blue, down-regulated genes (*n* = 156) (FDR < 0.05 and |log2FC| > 1). **(B)** Venn diagrams depicting the intersection between DEGs and the curated gene sets: 28 differentially expressed cellular-senescence-related genes (DE-CSRGs, top) and 24 differentially expressed metabolic-reprogrammingrelated genes (DE-MRRGs, bottom). **(C)** Strategy and numbers used to define differentially methylated CSRGs (DM-CSRGs, *n* = 8, top) and differentially methylated MRRGs (DM-MRRGs, *n* = 7, bottom) by overlapping methylation status (hyper/hypo) with expression direction (down/up). **(D)** Spearman correlation heat-map of the 15 overlapping differentially methylated CSRGs and MRRGs. Colour intensity indicates correlation coefficient (red, positive; blue, negative); only correlations with |r| > 0.3 and $p < 0.05$ are shown.)

LASSO regression and SVM-RFE were employed to identify key CSRGs and MRRGs among the 15 candidate genes. Of the 15 candidate genes, 6 genes, namely *GATM*, *SRM*, HNMT, *CXCL1*, *NDRG1*, and *SERPINE1*, were selected in the lambda.min model (Figure 5A). SVM-RFE with 10-fold cross-validation selected eight genes: *CXCL1*, *SRM*, *GATM*, *NDRG1*, *WSB1, SERPINE1*, *CYP1B1*, and *CCL2* (Figure 5B)). Finally, five overlapping genes from LASSO and SVM-RFE, namely, *CXCL1*, *SRM*, *GATM*, *NDRG1*, and *SERPINE1*, were identified as key CSRGs and MRRGs involved in CRC metastasis (Figure 5C)). *CXCL1* is involved in oncogene-induced senescence, whereas *SERPINE1* participates in replicative senescence. *SRM* has been implicated in glutathione, beta-alanine, arginine, proline, cysteine, and methionine metabolism. Additionally, *GATM* was involved in arginine and proline metabolism as well as glycine, serine, and threonine metabolism (Figure 5D)). Among these five genes, *CXCL1* and *SERPINE1* were predicted to be localized in the extracellular space, *SRM* in the cytoplasm, *GATM* in the mitochondria, and *NDRG1* in both the cytoplasm and nucleus (Table 1).

3.4. Key genes may affect the immune microenvironment and drug sensitivity of patients with CRLM

Liver metastasis, the leading cause of CRC-related mortality, is characterized by a highly heterogeneous and suppressive immune microenvironment (*11*). We compared immune cell infiltration profiles between the liver metastasis and primary tumor groups. The infiltration of MDSCs, natural killer cells, natural killer T cells, regulatory T cells, and type 1 T helper cells was significantly higher in the liver metastasis group (Figure 6A). Strong positive correlations were observed between *SERPINE1* and MDSCs (correlation coefficient, COR = 0.72), natural killer T cells (COR = 0.9), natural killer cells (COR = 0.82), regulatory T cells (COR = 0.85), and type 1 T helper cells (COR

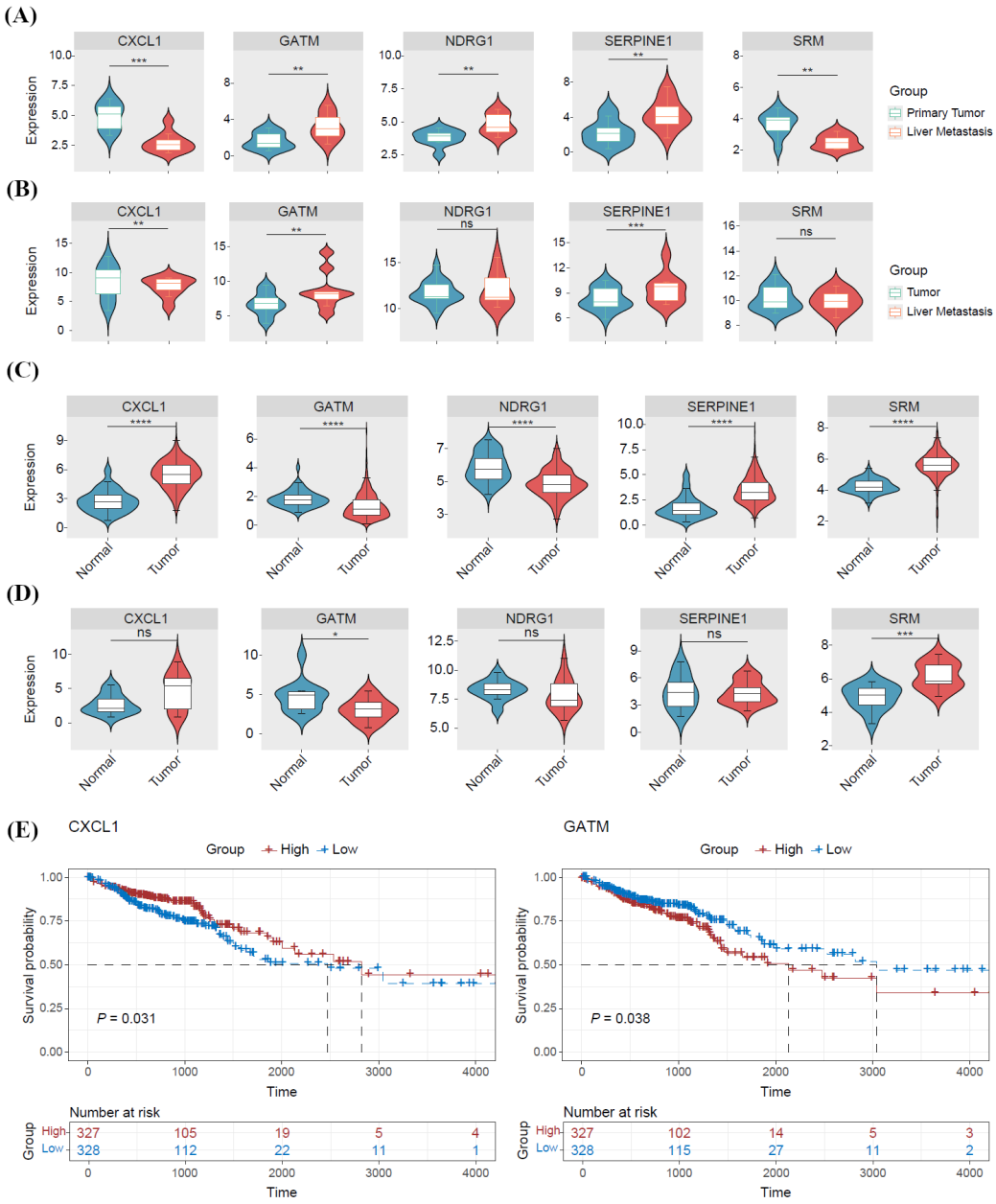

Figure 3. Transcript abundance and prognostic value of the five-gene senescence–metabolism signature. (A) Expression levels of *CXCL1*, *SERPINE1*, *NDRG1*, *SRM* and *GATM* in the discovery (GSE213402) cohort. Data are shown as fragments per kilobase of transcript per million mapped reads (FPKM) in primary tumours (n = 10) versus liver metastases (n = 10). P values were calculated with the paired Wilcoxon test. **(B)** RNA-seq validation of the same five genes in an independent set of 10 patient-matched fresh-frozen primary CRC and synchronous liver metastases collected at our centre (Huashan Hospital, 2014-2020). Read counts were normalized to TPM; boxes represent median ± interquartile range. **(C)** TCGA-COAD/READ dataset: box-plots comparing tumour (n = 607) versus adjacent normal mucosa (n = 51) for each signature gene. P values, Wilcoxon rank-sum test. **(D)** Huashan cohort: RNA-seq comparison of tumour versus adjacent normal mucosa (n = 10 pairs). P values, paired t test.**(E)** Kaplan-Meier plots of overall survival in TCGA-CRC patients stratified by median expression of *CXCL1* (left) and *GATM* (right). Log-rank P values are shown.

= 0.84) (Figure 6B). *SERPINE1* positively correlated with the immune functions of antigen-presenting cell co-stimulation (COR = 0.52), chemokine receptor signaling (COR = 0.59), immune checkpoint activity (COR = 0.55), parainflammation (COR = 0.67), T cell co-inhibition (COR = 0.55), and type 1 interferon response (COR = 0.61) (Figure 6C). *SERPINE1* was positively correlated with HLA-DQA2 (COR = 0.45), HLA-DQA1 (COR = 0.51), and HLA-DRB1 (COR = 0.46) (Figure 6D). These results suggest that *SERPINE1* is closely associated with the metastatic immune microenvironment. Variations in the immune microenvironment may influence drug sensitivities in patients with cancer. Thus, we compared sensitivity to chemotherapy between the metastatic and primary groups. A significant difference in the IC_{50} values was detected for the 30 therapeutic agents. Among them, oxaliplatin and 5-fluorouracil, which are commonly

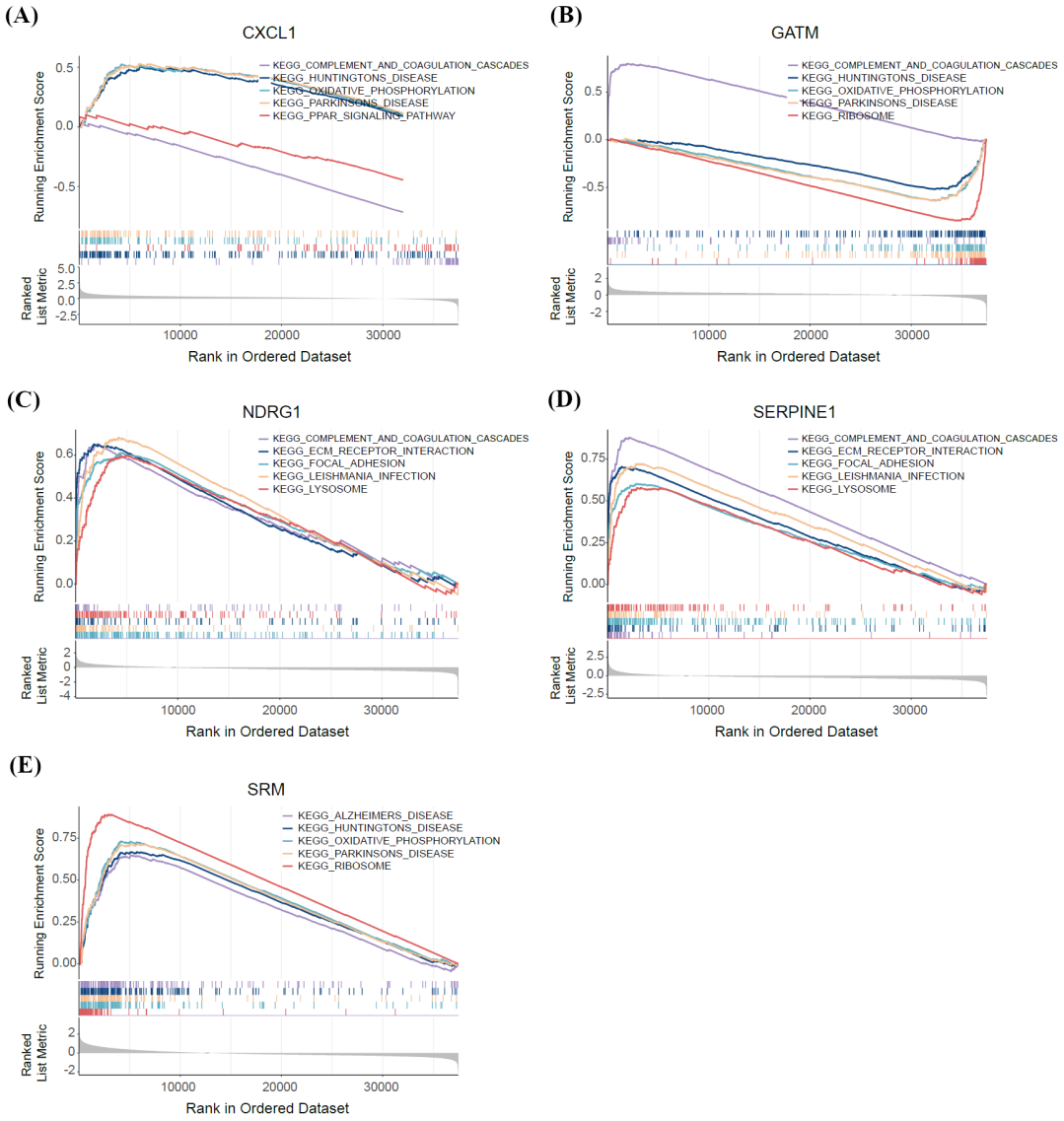

Figure 4. Gene-set enrichment analysis (GSEA) of the five-gene senescence–metabolism signature in CRLM. (A) *GATM* GSEA. Leading-edge subsets are enriched for ribosome, oxidative phosphorylation and Huntington-disease pathways. **(B)** *CXCL1* GSEA. Positive enrichment is observed for PPAR signalling, cytokine–cytokine receptor interaction and chemokine signalling pathways. **(C)** *NDRG1* GSEA. ECM-receptor interaction, focal adhesion and complement–coagulation cascades exhibit significant enrichment. **(D)** *SERPINE1* GSEA. Complement–coagulation cascade, ECM-receptor interaction and lysosomal pathways are positively enriched. **(E)** *SRM* GSEA. Oxidative phosphorylation, Parkinson-disease and ribosome pathways show significant enrichment.Genes were ranked by log2FC (metastasis vs primary tumour, x-axis; positive log2FC indicates upregulation in metastases); coloured lines trace the running enrichment score (y-axis) for the indicated pathways. All analyses used the c2.cp.kegg.v7.5.1.symbols.gmt gene set; FDR < 0.05 was considered significant.

used as chemotherapeutic agents for CRC, exhibited significantly higher IC_{50} values in the liver metastasis group than in the primary tumor group (Figure 7A). Furthermore, *SRM* expression was negatively correlated with IC_{50} values of 5-FU (r = -0.55), indicating that high *SRM* expression is associated with increased sensitivity to 5-FU. *SRM* negatively correlated with oxaliplatin levels (r = -0.45). Conversely, *NDRG1* positively correlated with oxaliplatin levels (r = 0.46), whereas *CXCL1* showed a negative correlation (r = -0.45) (Figure 7B). These results suggest the involvement of key genes in the mechanisms relevant to chemotherapeutic resistance in CRC metastasis.

4. Discussion

CRLM remains the dominant cause of death in patients with CRC; however, the molecular programs that allow disseminated tumor cells to survive, adapt, and ultimately colonize the hepatic parenchyma remain poorly understood. By integrating genome-wide DNA methylation profiles with bulk RNA sequencing data and curated gene sets for cellular senescence and metabolic reprogramming, we identified a five-gene epigenetic–transcriptomic signature comprising *CXCL1*, *SERPINE1*, *NDRG1*, *SRM*, and *GATM*, which functionally couples senescence-associated secretory traits with

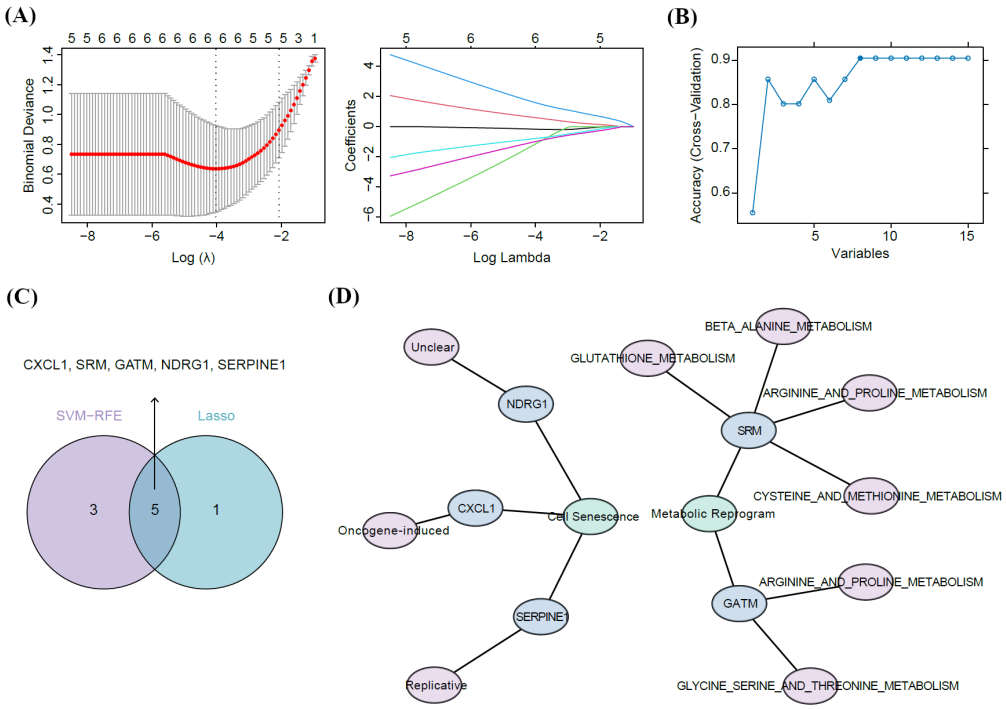

Figure 5. Machine-learning identification of a five-gene senescence–metabolism signature predictive of CRLM. (A) Left: Ten-fold cross-validated binomial deviance (y-axis) plotted against log(λ) (x-axis) for LASSO logistic regression of 15 candidate DM-CSRGs/DM-MRRGs. Red dots indicate mean deviance ± 1 SE; vertical dashed lines mark λ_min (left) and λ_1SE (right). Right: Profile of regression coefficients (y-axis) versus log(λ) (x-axis); each coloured line represents one gene, illustrating coefficient shrinkage with increasing penalty. Six genes (*GATM*, *SRM*, *HNMT*, *CXCL1*, *NDRG1*, *SERPINE1*) survived at λ_min. **(B)** SVM-RFE recursive feature elimination. Mean cross-validation accuracy (y-axis) is plotted as a function of the number of input genes (x-axis). The peak accuracy (eight genes) is indicated by the red dot; the selected subset comprises *CXCL1*, *SRM*, *GATM*, *NDRG1*, *WSB1*, *SERPINE1*, *CYP1B1* and *CCL2*. **(C)** Venn overlap of genes retained by LASSO (purple) and SVM-RFE (blue) yielding the final five-gene signature: *CXCL1*, *SERPINE1*, *NDRG1*, *SRM* and *GATM*. **(D)** Functional classification of the five signature genes according to their primary roles in cellular-senescence pathways (*CXCL1*, *SERPINE1*, *NDRG1*) or metabolic reprogramming (*SRM*, *GATM*).

Table 1. Subcellular localization prediction of the five hub genes

Gene symbol	Predicted primary localization	Prediction score	Experimental evidence (UniProt)
CXCL1	Extracellular	0.91	Secreted chemokine (P09341)
SERPINE1	Extracellular	0.89	Secreted serpin (P05121)
SRM	Cytoplasm	0.83	Soluble cytoplasmic enzyme (P19623)
GATM	Mitochondrion	0.79	Mitochondrial matrix protein (P50440)
NDRG1	Cytoplasm / Nucleus	0.76 / 0.71	Dual-localized scaffold protein (Q92597)

ND: Predictions were generated with Cell-Ploc 2.0 (*http://www.csbio.sjtu.edu.cn/bioinf/Cell-Ploc-2/*). Scores represent the highest voting confidence among 11 integrated algorithms.

metabolic plasticity in CRLM. The global shift toward hypomethylation observed in liver metastases (66,975 hypomethylated versus 31,883 hypermethylated CpGs) recapitulates previous reports describing widespread methylation erosion during metastatic progression (*12*) and supports the concept that the loss of DNA methylation fidelity facilitates chromosomal instability and enhancer activation (*13*). Pathway-level annotation has consistently implicated the PI3K–Akt, MAPK, and ECM-receptor interaction cascades, all of which are linked to CRC cell extravasation and hepatic colonization (*14-16*).

CXCL1, *SERPINE1*, *NDRG1*, *SRM*, and *GATM* have been implicated in discrete aspects of CRC biology; however, their concerted actions in CRLM remain unknown. *CXCL1* and *SERPINE1* are canonical SASP factors that sustain neutrophil- and MDSC-rich niches in the metastatic liver and were associated with focal hypomethylation, echoing prior reports of NF-κB- and TGF-β-driven chemokine induction during oncogene-induced senescence (*6*). *NDRG1*, traditionally viewed as a hypoxia-responsive suppressor, was demethylated and overexpressed in metastases, consistent with recent data linking its antioxidant function to oxaliplatin resistance (*17*). The metabolic enzymes *SRM* and *GATM*, although seldom studied in CRC, control polyamine and creatine–

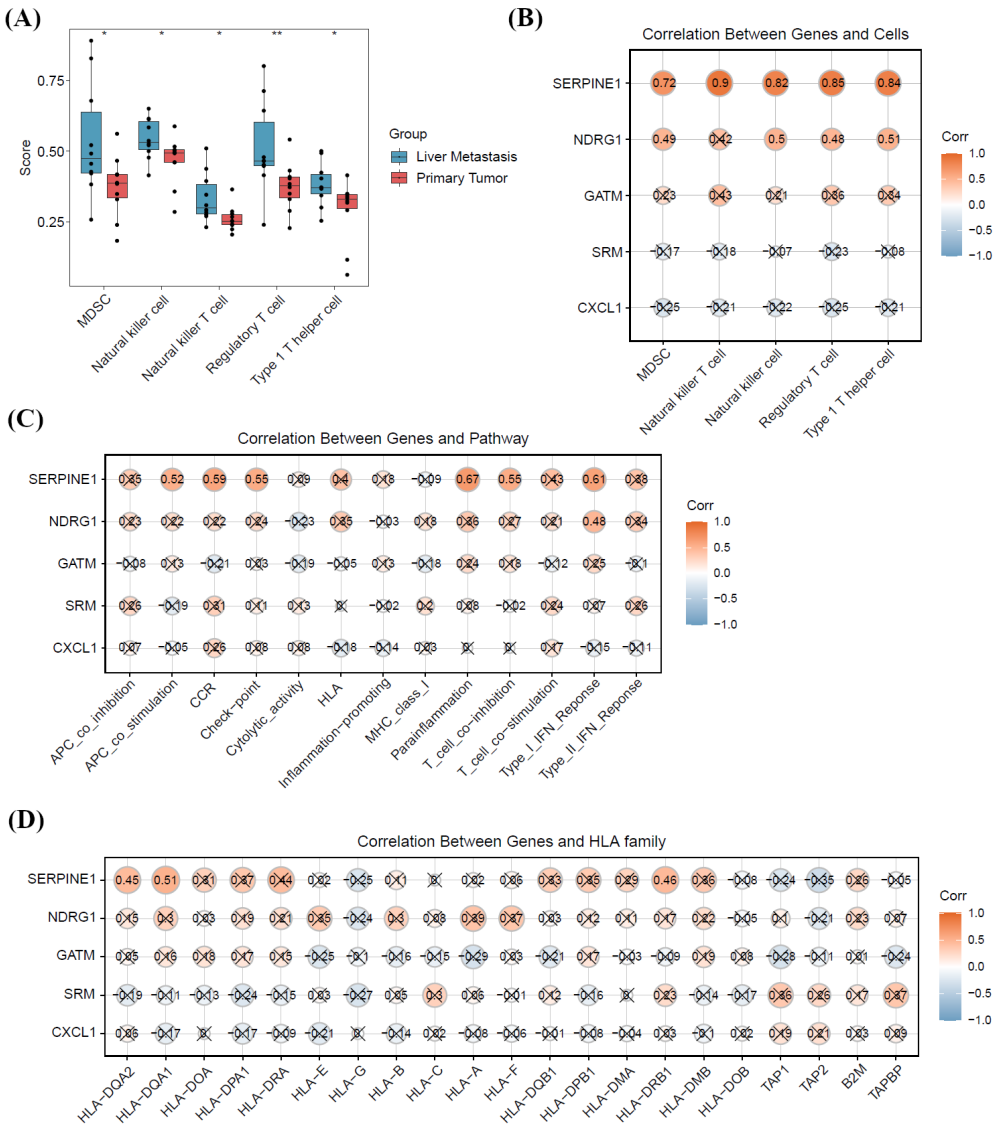

Figure 6. Immune landscape linked to the five-gene senescence–metabolism signature in CRLM. (A) Box-plots comparing the infiltration scores of 28 immune cell types between liver metastasis and primary tumour samples (GSE213402). Only significantly altered populations are shown (Wilcoxon test, FDR < 0.05). MDSC, myeloid-derived suppressor cell; NK, natural killer; Treg, regulatory T cell; Th1, type-1 T helper cell. **(B)** Spearman correlation heat-map between the five signature genes and the differentially infiltrated immune cells. Colour intensity reflects correlation coefficient (red, positive; blue, negative); asterisks indicate FDR < 0.05. **(C)** Correlation of signature genes with immune-related functions: antigen-presenting cell co-stimulation, chemokine receptor signaling, immune checkpoint activity, parainflammation, T-cell co-inhibition, and type I interferon response, as well as with HLA gene expression. Circle size and colour scale represent absolute correlation coefficient; only $|r| > 0.3$ and FDR < 0.05 are plotted. **(D)** Spearman correlation matrix between the five signature genes and HLA-family genes in the GSE213402 cohort. Colour intensity indicates correlation coefficient; asterisks denote FDR < 0.05.

phosphocreatine flux, respectively, and their elevated expression aligns with the dependence of disseminated tumor cells on de novo polyamine synthesis and mitochondrial ATP buffering (*18,19*). Collectively, these five genes appear to link the two hallmarks of CRLM — senescence bypass and metabolic reprogramming — and provide readily testable biomarkers for therapeutic stratification.

The GSEA profile of our five-gene signature was dominated by four functional modules: complement and coagulation cascades, focal adhesion/ECM-receptor interactions, OXPHOS, and PPAR signaling, each of which has been independently implicated in CRLM (*11,14*). Complement–coagulation axis activation is one of the most recurrent signatures in prior transcriptomic surveys and has recently been validated functionally; deletion of factor B of the alternative complement pathway reduced liver tumor burden by >60% in a syngeneic CRLM model (*20*). The strong enrichment of *SERPINE1* and *CXCL1* within this cascade is consistent with the hypothesis that SASP factors may contribute to attracting myeloid cells and, potentially, to propagating a fibrin-rich metastatic niche *via* complement amplification (*6,21,22*). Focal-adhesion/ECM-receptor genes were

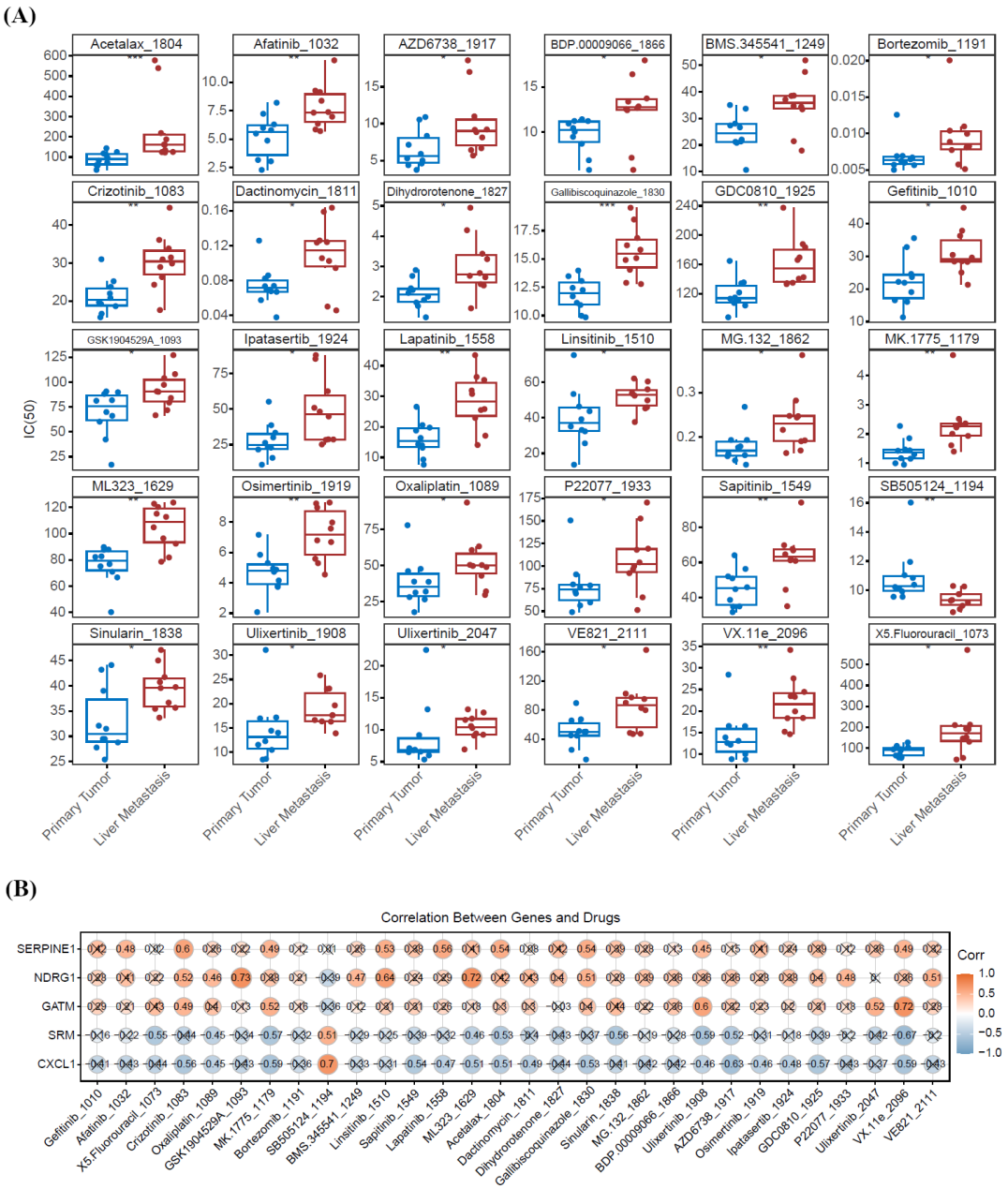

Figure 7. Chemotherapy sensitivity linked to the five-gene senescence–metabolism signature in CRLM. (A) Box-plots comparing predicted IC_{50} values of commonly used chemotherapeutic agents between primary tumour and liver-metastasis samples (GSE213402). IC_{50} values were calculated with the oncoPredict algorithm; central line indicates median, whiskers 1.5 × IQR. *P* values, paired Wilcoxon test. **(B)** Spearman correlations between the five signature genes and IC_{50} values of commonly used chemotherapeutic agents. Significant negative correlations with *SRM* and positive correlations with *NDRG1* are observed ($|r| > 0.4$, FDR < 0.05). Negative correlation indicates increased drug sensitivity.

previously identified as hub nodes in a 321-gene CRLM network and correlated with poor survival (*23*). Our observation that *NDRG1* and *SERPINE1* are enriched within the ECM-receptor signature raises the hypothesis that these cancer-cell-intrinsic programs may contribute to ECM remodeling during intrahepatic colonization (*17,24*). Finally, the co-enrichment of OXPHOS and PPAR signaling mirrors single-cell data showing that both tumor cells and lipid-associated TAMs upregulate mitochondrial respiration and PPARγ activity in CRLM (*25*). The positioning of *SRM* and *GATM* within these metabolic gene sets suggests a potential — yet still unproven — mechanistic connection between epigenetic senescence bypass and bioenergetic adaptation (*26*), offering testable targets for metabolic–immune combination therapy in CRLM (*9,27*).

Our comparative profiling of liver metastases versus primary tumors revealed a coherent, stepwise reprogramming that appears to converge across the transcriptional, immune, and pharmacological layers, yielding immediately testable clinical hypotheses. First, the five-gene senescence–metabolic hub (*CXCL1*,

SERPINE1, *NDRG1*, *SRM*, and *GATM*) is consistently upregulated in metastases and is driven by locus-specific hypomethylation rather than by copy-number gain, implying that epigenetic therapy (*e.g.*, low-dose DNMT inhibitors) or biological neutralization of *CXCL1*/PAI-1 represents a testable — yet still hypothetical — strategy to reverse the metastatic phenotype attributed to the five-gene hub (*28*). Notably, CXCL1 and SRM were downregulated in liver metastases compared to primary tumors (GSE213402), yet upregulated in primary tumors compared to normal mucosa (TCGA). This apparent contradiction may reflect stage-specific roles of these genes during tumor evolution — early activation during tumorigenesis, followed by transcriptional suppression in the metastatic niche, potentially driven by microenvironmental cues or epigenetic reprogramming. Second, the parallel enrichment of MDSCs and Tregs, together with the altered abundance of NK cells and the downregulation of HLA-DQA1/DRB1, indicates that immune evasion may be orchestrated by the same hub genes; pre-clinical models are needed to examine whether targeting the polyamine–creatine axis (*e.g.*, DFMO or *GATM* inhibitors) can impair tumor growth, enhance antigen presentation, and potentially synergize with PD-1/LAG-3 blockade (*29*). Finally, the inverse correlation between *SRM* expression and 5-FU/oxaliplatin IC_{50}, in contrast to the positive correlation with *NDRG1*, generates the hypothesis that these genes might inform chemotherapy selection; prospective clinical validation is essential before any patient-triage application(*30*). Collectively, our bioinformatic analyses generate the hypothesis that the five-gene signature could guide decision-making for combined metabolic-immune chemotherapy in CRLM; functional and clinical studies are now required to confirm its utility.

In summary, our five-gene signature integrates senescence bypass, metabolic reprogramming, and immune evasion in CRLM, and provides potential biomarkers for patient stratification and combination therapy. Due to the limited sample size of the GSE213402 cohort (n = 10 pairs), our findings are exploratory and require validation in larger independent cohorts. Machine-learning-derived signatures may be prone to overfitting at this scale. Functional assays and prospective clinical validation are warranted to confirm the causal roles of the five-gene signature in CRLM progression and therapy response.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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Original Article

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Effect of Yiqi Bushen Shugan Huoxue Decoction on impaired endometrial receptivity associated with ovarian stimulation: A clinical trial, modular pharmacology, molecular docking, and experiment-based study

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SUMMARY: Endometrial receptivity plays a critical role in pregnancy, while controlled ovarian hyperstimulation (COH) — widely used for infertile patients — could impair endometrial receptivity and subsequent pregnancy outcomes. This study aims to explore the effect of Yiqi Bushen Shugan Huoxue Decoction (YBSHD) on impaired endometrial receptivity in patients with unexplained infertility (UI) undergoing COH and to determine the mechanism for it through modular pharmacology, molecular docking, and a murine model. First, we retrospectively studied 422 patients with UI who underwent COH to get pregnant. Results indicated that the live birth rate significantly increased in the YBSHD group. Second, a systematic network pharmacology analysis was performed to screen the ingredients and possible targets of YBSHD. The main targets concerning YBSHD and endometrial receptivity involved pathways including hormone regulation, inflammatory responses, and apoptosis. The active components of quercetin and kaempferol from YBSHD exhibited a strong binding affinity to key molecules, including BCL2, ESR1, IL6, IL1B, and TNF. Third, YBSHD improved endometrial receptivity in a murine COH model. Compared to the COH group, the number of embryo implantations and endometrial pinopodes significantly increased in the YBSHD group, indicating improved endometrial receptivity. YBSHD improved the local immune microenvironment in COH mice by regulating excessive hormone secretion, gene expression of inflammatory factors, and proportions of neutrophils and macrophages. Moreover, YBSHD inhibited apoptosis in the ovaries and uteruses of COH mice. In summary, YBSHD could increase the live birth rate in patients with UI, mainly because it can inhibit inflammation and cell apoptosis, thereby improving endometrial receptivity.

Keywords: endometrial receptivity, traditional Chinese medicine, modular pharmacology, inflammation, apoptosis

1. Introduction

Studies have found that the lifetime prevalence and period prevalence of infertility have reached 17.5% and 12.6%, respectively (*1,2*). Despite the development of *in vitro* fertilization embryo-transfer technology (IVF-ET), the clinical pregnancy rate and live birth rate remain around 30% worldwide (*3-5*). A major reason is the failure of the embryo to implant in a receptive uterus. Successful embryo implantation occurs during the synchronization of the embryo and the endometrium. However, controlled ovarian hyperstimulation (COH), commonly used for infertile women, may impair endometrial receptivity by altering the release of hormones and the window of implantation.

Various means of enhancing endometrial receptivity have been tested from bench to bedside, including aspirin (*6*), sildenafil (*7*), and endometrial scratching (*8*). However, the guidelines have yet to reach a definitive conclusion to date. Chinese herbal medicine has demonstrated unique advantages in dealing with poor endometrial receptivity in recent years. Current clinical

methods used to manage it include addressing a Kidney deficiency, Liver Qi stagnation, and a Spleen deficiency (*9,10*). The herbal formula Yiqi Bushen Shugan Huoxue Decoction (YBSHD), consisting of 18 precisely selected herbs, was formulated in line with traditional Chinese medicine (TCM) theory and the clinical experience of the present research team. Our clinical observations indicated that COH could adversely affect pregnancy outcomes, whereas emerging evidence suggests that YBSHD has the potential to enhance endometrial receptivity in infertile patients. Therefore, a clinical trial involving patients with unexplained infertility (UI) undergoing COH and a murine COH model were designed to explore the effect and mechanism of YBSHD on impaired endometrial receptivity caused by COH. Moreover, network pharmacology analysis and molecular docking were used to explore the mechanism of YBSHD. The flowchart for this study is shown in Figure 1. By establishing a comprehensive "YBSHD-active components-therapeutic targets-endometrial receptivity" network, the study has sought to provide scientific validation for the use of this traditional formulation in contemporary reproductive medicine.

2. Materials and Methods

2.1. A retrospective clinical trial

2.1.1. Subjects

Patients with UI who were seen at the Obstetrics and Gynecology Hospital of Fudan University between January 2018 and January 2022 were retrospectively identified. The study was approved by the ethics committee of this hospital (kyy2020-156), and this study was conducted in accordance with the Helsinki Declaration.

2.1.2. Criteria

Subjects were patients with UI who were unable to conceive after at least one year of sexual intercourse without contraception. Inclusion criteria included: 1) between 20 and 40 years of age; 2) body mass index (BMI) < 30 kg/m^2; 3) no tubal infertility according to hysterosalpingography or ultrasound; 4) no ovulation abnormalities; 5) the spouse's semen was within the normal range; 6) couples with a normal chromosome karyotype; 7) patients undergoing COH. Exclusion criteria included: 1) a congenital uterine malformation; 2) endometrial diseases such as intrauterine adhesions, endometrial polyps, thin endometrium, endometrial hyperplasia, endometritis, or endometrial tuberculosis; 3) submucosal fibroids, intramural fibroids, moderate or severe endometriosis, or adenomyosis; 4) endocrine and metabolic disorders; 5) autoimmune diseases; and 6) malignant tumors or other serious diseases. Patients with UI were selected by excluding those with evident causes of infertility or endometrial pathologies. Known confounders affecting endometrial receptivity were controlled for, enabling the evaluation of YBSHD's impact on COH-associated endometrial dysfunction in patients with UI.

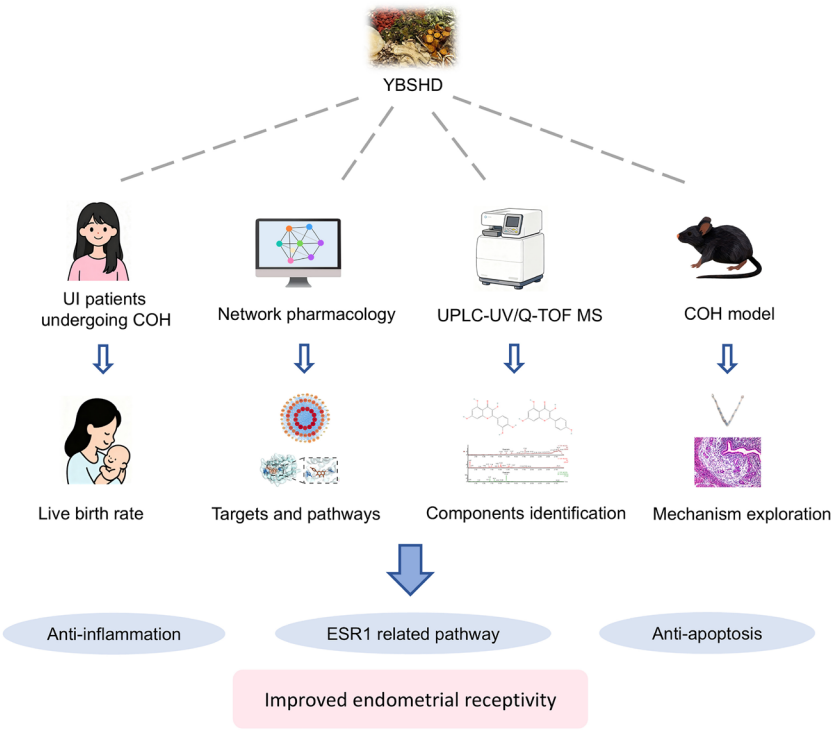

Figure 1. Flowchart of the study.

2.1.3. Intervention

Four hundred and twenty-two patients with UI were divided into a YBSHD group and a control group according to their medication regimen. The dosage regimen for COH for all patients was based on relevant clinical trials (*11,12*). Patients in the control group were administered letrozole 2.5 mg once a day for 5 consecutive days from the third to the fifth day of the menstrual cycle. Based on the control group, patients in the YBSHD group were administered both letrozole and YBSHD, the herbal components of which are listed in Supplementary Table S1 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=278*), at the same time. YBSHD was administered orally at a dose of 150 ml twice a day for 14 days. For patients in all groups, 75 IU of human menopausal gonadotropin (HMG) was administered once daily for another 5 days after letrozole treatment. Follicles were monitored from the 10th to the 12th day of the menstrual cycle. Ten thousand IU of human chorionic gonadotropin (hCG) was injected when the dominant follicle reached 18 mm in diameter. Then, the patient was instructed to have sexual intercourse three times every other day. Dydrogesterone (10 mg, twice a day) was administered continuously for 10 days from the 15th to the 20th day of the menstrual cycle. Patients in both groups were treated for 3 consecutive menstrual cycles. The medication was discontinued if pregnancy was confirmed.

2.1.4. Outcomes

The primary outcomes included the clinical pregnancy rate and live birth rate. Definitions were based on the WHO guidelines and international consensus (*13,14*). Clinical pregnancy was defined as the presence of a gestational sac in vaginal ultrasound at six weeks of gestation. Live birth was defined as the delivery of a live fetus after 20 weeks of pregnancy. The evaluation of safety included liver and kidney function tests, routine blood tests, a routine stool examination, and routine urine tests.

2.2. Network pharmacology and fingerprint analysis

Methods for identifying the components and targets of YBSHD, prediction of the compound-target relationship network, protein-protein interaction (PPI) network and enrichment analysis, molecular docking, and fingerprint analysis of YBSHD are shown in the Supplementary Data (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=278*).

2.3. Animals and treatments

All C57BL/6 mice were purchased from Shanghai Jiesijie Experimental Animal Technology Co., Ltd. Female mice between the ages of 4 weeks and 6 weeks and male mice between the ages of 6 weeks and 8 weeks were used. The mice were housed in a specific pathogen-free environment (light/dark cycle, 12/12 h; ambient temperature, 20-25°C) and fed a regular chow diet ad libitum. All procedures conformed to ethical regulations and were approved by the Ethics Committee of the Hospital of Obstetrics and Gynecology, Fudan University. Mice were randomly divided into three groups (Ctrl group, COH group, and YBSHD group) based on weight. The workflow of the animal experiment is summarized in Figure 5A. The Ctrl and COH groups were gavaged with distilled water (0.2 ml/20 g bw/d) from day 1 to day 10. The YBSHD group was gavaged with an equal volume of YBSHD (29.3 mg/g bw/d) during the same period. Decoction products of YBSHD were obtained from the pharmacy of the Hospital of Obstetrics and Gynecology, Fudan University. They were concentrated and sterilized with a rotary evaporator and stored in a -20°C refrigerator for future use. Both the COH group and YBSHD group received intraperitoneal injections of PMSG (10 IU/0.1 ml, ProSpec Technogene, Israel) on day 7, followed by hCG (10 IU/0.1 ml, ProSpec Technogene, Israel) 48 h later. 0.1 ml of saline was given intraperitoneally to the Ctrl group simultaneously. Then, the female and male mice were mated at a ratio of 1:2 or 2:3 overnight. The morning of vaginal plug formation was counted as pregnant day (PD) 0.5. Mice were anesthetized with tribromoethanol (0.2 ml/10 g, Aladdin, China) and killed by cervical dislocation at PD 4.5 for tissue processing. Materials and methods for analysis of embryo implantation, H&E staining, scanning electron microscopy, ELISA of serum hormones, RT-qPCR, flow cytometric profiling of murine cells, and TUNEL staining are shown in the Supplementary Data (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=278*).

2.4. Statistical analysis

Methods for statistical analysis are shown in the Supplementary Data (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=278*).

3. Results

3.1. Effect of YBSHD on pregnancy outcomes in patients with UI undergoing COH

Potential subjects for the retrospective study were 656 patients with UI, 508 of whom met the criteria. Exclusions due to non-compliance with medical advice and loss to follow-up resulted in a final analysis of 214 controls and 208 patients receiving YBSHD, for a total of 422 patients (Supplementary Figure S1, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=278*). The demographic baseline characteristics

of the patients were analyzed and compared. The mean age was 31.4 ± 3.7 and 30.7 ± 4.0 for the control group and the YBSHD group (P = 0.06). Years of infertility were similar in the two groups (3.2 ± 2.1 versus 3.0 ± 1.9; P = 0.69). There were no significant differences between the two groups in terms of BMI (21.0 ± 5.7 versus 21.2 ± 4.5; P = 0.31).

The pregnancy outcomes were further analyzed in patients with UI. The clinical pregnancy rate was 26.17% in the control group and 32.69% in the YBSHD group, but the difference was not significant. The live birth rate was 21.03% in the control group. Compared to the control group, the live birth rate of 29.81% in the YBSHD group was significantly higher, suggesting that YBSHD effectively improves endometrial receptivity and promotes live births in patients with UI undergoing COH (Table 1). The safety of YBSHD was evaluated during the treatment and follow-up periods. Adverse reactions in the patients included nausea, vomiting, and mild abdominal pain. No serious drug adverse reactions were reported.

Table 1. Pregnancy outcomes of patients with UI

	Control group (n = 214)	YBSHD group (n = 208)	P value
Clinical pregnancy rate (%)	56 (26.17)	68 (32.69)	0.16
Live birth rate (%)	45 (21.03)	62 (29.81)	0.04

YBSHD, Yiqi Bushen Shugan Huoxue Decoction.

3.2. Identification of active ingredients and targets of YBSHD

The active ingredients of YBSHD and their corresponding targets were obtained using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database (Supplementary Table S1, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=278*). The most common compounds sorted by topological degree were as follows: quercetin (in 9 herbs, degree=140), kaempferol (in 9 herbs, degree = 59), luteolin (in 4 herbs, degree = 55), β-sitosterol (in 7 herbs, degree=37), and stigmasterol (in 8 herbs, degree = 3) (Supplementary Table S2, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=278*). The target information was matched with the protein target information in the UniProt database, resulting in 224 compounds and 310 targets after deduplication. A total of 3327 compound-target relationships were visually depicted as a compound-target network by Cytoscape (Figure 2A).

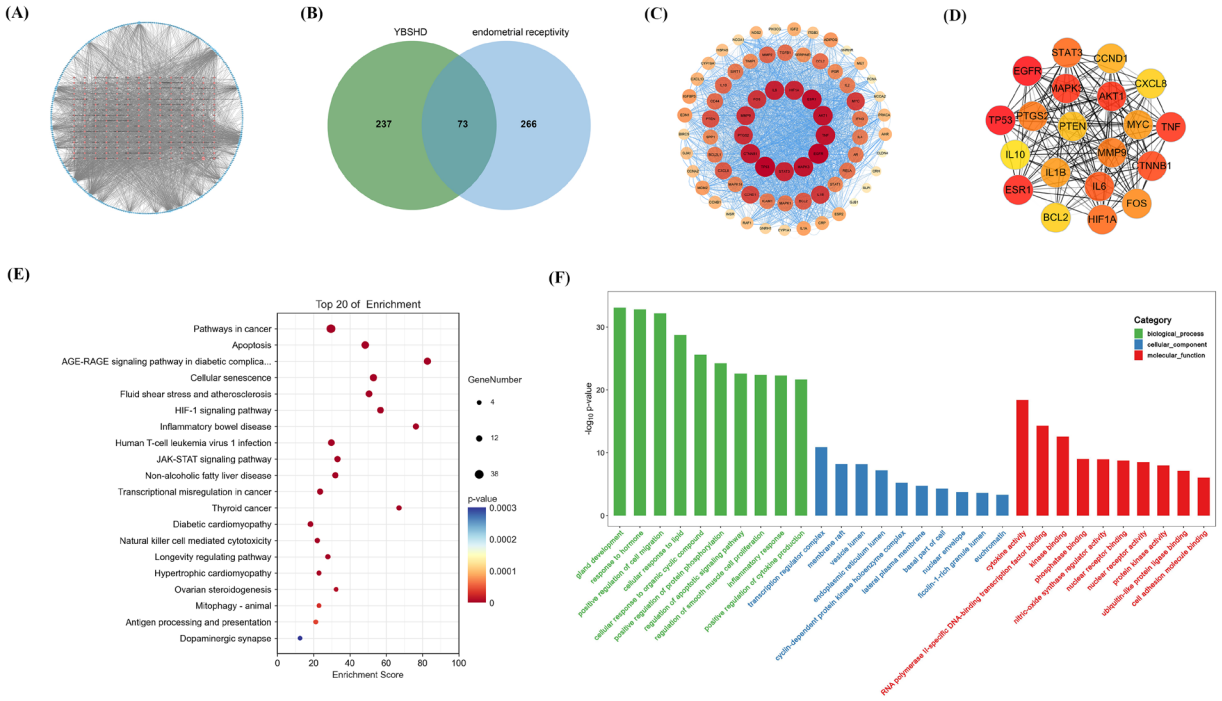

Figure 2. Network target analysis of YBSHD's effect on endometrial receptivity. (A) The network showed the compound-target relationship of YBSHD. Pink and blue nodes represent the compound and target genes of YBSHD, respectively. **(B)** The Venn diagram highlighted 73 overlapping targets between YBSHD and endometrial receptivity. **(C)** PPI network analysis of YBSHD targets and endometrial receptivity. The nodes represent target proteins, and the edges represent protein-protein associations. The node colors range from red to yellow, and sizes range from large to small, representing the degree of protein binding. **(D)** The top 20 targets network generated by Cytoscape. The nodes represent the core target genes, and the edges represent the interactions between targets. The node's color, which ranges from red to yellow, represents the degree of targets in descending order. **(E)** Bubble graph of the KEGG pathway for the top 20 core genes. **(F)** GO analysis showed therapeutic targets in biological processes, cellular components, and molecular functions.

3.3. Network analysis of YBSHD's effect on endometrial receptivity

Six hundred and seventy-eight targets related to endometrial receptivity were identified from the GeneCards, DrugBank, and OMIM databases, and 339 targets were finally determined based on relevance. A Venn diagram revealed 73 overlapping targets between the 339 endometrial receptivity-related targets and the 310 drug targets from YBSHD (Figure 2B). The 73 targets were imported into the String database to obtain a PPI network of YBSHD and endometrial receptivity, and the result was graphically depicted using Cytoscape (Figure 2C). The top 20 target genes, including TNF, ESR1, AKT1, HIF1A, BCL2, CXCL8, EGFR, TP53, MAPK3, CTNNB1, IL6, STAT3, PTGS2, MMP9, FOS, MYC, IL1B, CCNBD1, PTEN and IL10, were identified with the plugin cytoHubba (Figure 2D and Supplementary Table S3, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=278*).

3.4. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analysis

KEGG pathway and GO enrichment analysis were performed using the top 20 target genes identified. KEGG analysis indicated that the pathways associated with the regulation of YBSHD in endometrial receptivity mainly involve apoptosis of cells, JAK-STAT signaling pathway, ovarian steroidogenesis, mitophagy, antigen processing and presentation, *etc.* (Figure 2E). The biological processes involved in the GO enrichment analysis include inflammatory response, response to hormones, positive regulation of cell migration, regulation of apoptotic signaling pathways, positive regulation of cytokine production, and gland development. Cellular components involved in the GO enrichment analysis include transcription regulator complex, endoplasmic reticulum lumen, and nuclear envelope. The molecular functions involved include cytokine activity, phosphatase binding, nuclear receptor binding, nuclear receptor activity, cell adhesion and molecule binding (Figure 2F).

3.5. Molecular docking of quercetin and kaempferol

According to network pharmacology, the main ways for YBSHD to improve endometrial receptivity may be related to the response to hormone, regulation of apoptotic signaling pathway, and inflammatory response. Results also indicated that quercetin and kaempferol were the top two components in YBSHD in terms of degree and the number of single herbs containing these components. Therefore, quercetin and kaempferol were selected as the primary active components of YBSHD, and their potential binding affinity to those related targets (ESR1, BCL2, IL6, IL1B, and TNF) was evaluated using molecular docking (Figure 3). The general belief is that the absolute value of the docking score >5 kcal/mol denotes favorable binding, whereas an absolute value >9 kcal/mol implies exceedingly strong binding activity. Therefore, molecular docking further verifies the advantage of YBSHD compounds in acting on multiple targets and pathways. Quercetin and kaempferol from YBSHD demonstrated strong binding affinity with ESR1, BCL2, IL6, IL1B, and TNF (Table 2).

3.6. Analysis of the chemical composition of YBSHD

The above results confirmed the clinical efficacy of YBSHD in treating patients with UI. Network pharmacology suggested that YBSHD may act through multiple pathways, with quercetin and kaempferol (chemical structure shown in Figure 4A-B) likely serving as key components. Here, fingerprint analysis was used to identify the active ingredients of YBSHD. UPLC-UV/Q-TOF MS technology revealed the chromatographic fingerprint of YBSHD (Figure 4E). The mass spectrum of quercetin and kaempferol standards showed a detectable peak at a mass-to-charge ratio (*m/z*) of approximately 302 and 286, respectively (Figure 4C-D), which matches the molecular weight, suggesting that the mass spectra of the standards can serve as a reference for fingerprint analysis. The mass spectrum of YBSHD was compared to those of the two reference standards, and the results indicated that the chromatogram of YBSHD contained both quercetin and kaempferol peaks, confirming the presence of these two components in YBSHD.

3.7. Effect of YBSHD on endometrium and embryo implantation in COH mice

One of the pivotal indicators of decreased endometrial receptivity is the reduction in embryo implantation (*15*). Consistent with the clinical investigation of YBSHD in patients with UI undergoing COH, a COH mouse model was used to verify YBSHD's effect on endometrial receptivity. The implantation sites in each group were counted by injecting a Chicago sky blue dye solution intravenously into PD 4.5 mice. Results indicated that the mean number of implantation sites decreased significantly in the COH group compared to that in the Ctrl group, with observable uterine swellings and hydrops in the uterine horns in the COH group, suggesting a hyperstimulated state (Figure 5B). YBSHD treatment increased the number of embryo implantations (Figure 5F) and restored uterine morphology. There were no significant differences in the uterus index among the groups (Figure 5G).

Endometrial morphology was further observed using H&E staining. Compared to the Ctrl group, mice in the COH group had a significantly thinner endometrium and fewer glands, characterized by small glandular lumens with insufficient secretion and a more compact stroma

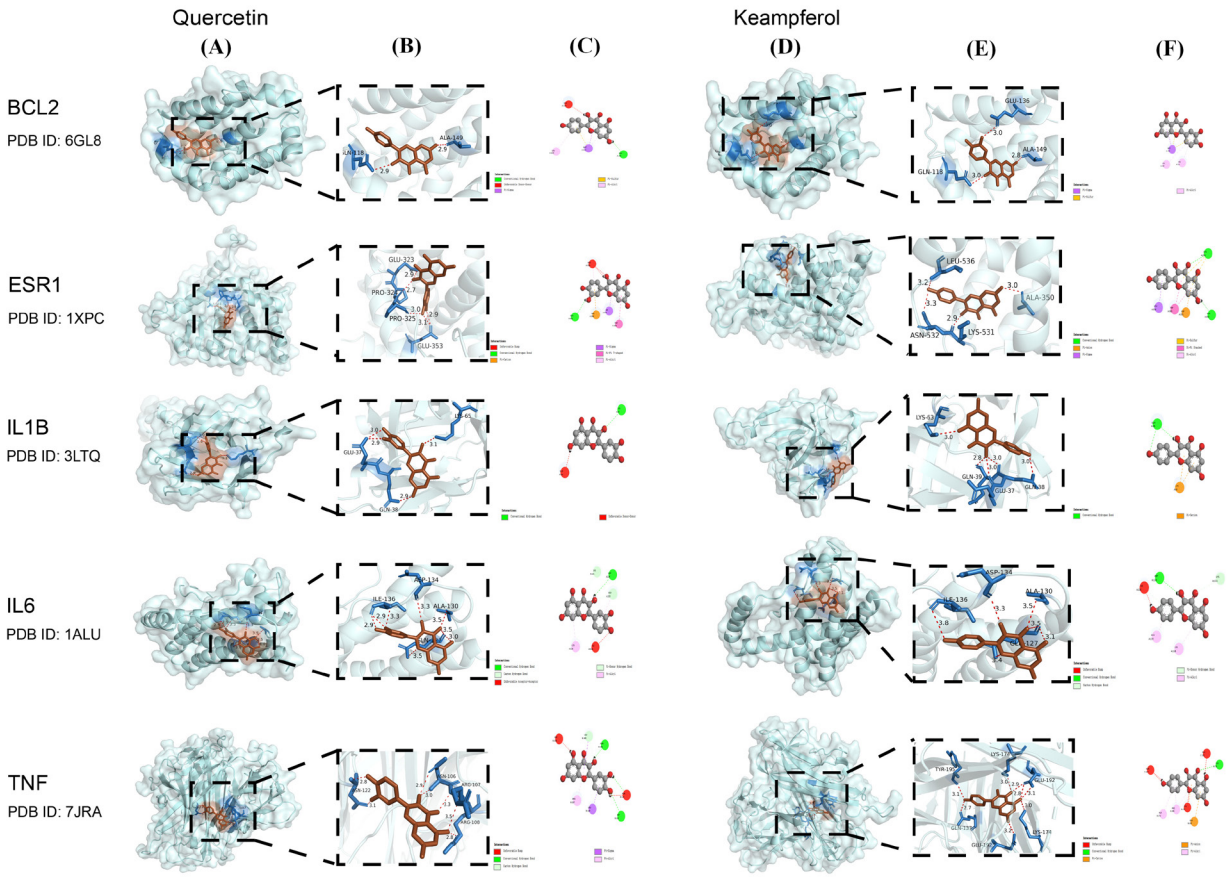

Figure 3. Molecular docking of quercetin and kaempferol with five gene proteins. Quercetin **(A)** / kaempferol **(D)** is located in the protein cavity pocket. Quercetin **(B)** / kaempferol **(E)** forms hydrogen bonds with key amino acid residues of proteins. Red dotted lines denote hydrogen bonds. Quercetin **(C)** / kaempferol **(F)** and protein 2D structure diagram. Green dotted lines represent hydrogen bonds. Pi-pi interaction is depicted with pink dotted lines, and pi-cation and pi-anion interactions are depicted with orange dotted lines.

Table 2. Molecular docking results of quercetin and kaempferol with five target genes

Component	Affinity (kcal/mol)				
	TNF	ESR1	IL6	IL1B	BCL2
Quercetin	-5.9	-7.2	-5.7	-5.0	-6.8
Kaempferol	-8.7	-8.3	-6.4	-6.3	-7.8

(Figure 5C-D). YBSHD administration thickened the endometrium and restored the number and size of uterine glands (Figure 5H-I). The enlarged lumen contained secretory substances, which displayed a secretory phase change. The stroma became loose and gradually differentiated into decidual cells after being treated with YBSHD. Pinopodes are a specific morphological marker of endometrial receptivity. The luminal surface of the endometrial epithelia in the COH mice was relatively smooth and flat, and the membrane projections were sparse with irregular arrangement and margin compared to the Ctrl group (Figure 5E). The reduction of pinopodes in COH mice indicated a lagged development of pinopodes. In mice treated with YBSHD, the dome-shaped bulge re-emerged on the endometrial epithelial surface and was covered with microvilli. An increased number of pinopodes confirmed improved endometrial receptivity in YBSHD mice (Figure 5J).

3.8. Effect of YBSHD on hormone receptors in COH mice

Estrogen and progesterone play a crucial role in the establishment of endometrial receptivity by regulating endometrial growth and development. Both hormones perform diverse functions mainly through binding to their corresponding receptors. Accordingly, the levels of expression of estrogen receptor 1 and progesterone receptor in the uterus were examined. Results confirmed that the expression of *Esr1* and *Pr* mRNA was significantly down-regulated in the COH model (Figure 5K-L). In contrast, YBSHD treatment significantly up-regulated the expression *of Esr1* compared to that in the COH group (Figure 5K). Leukemia inhibitory factor (LIF), one of the classic markers of endometrial receptivity, is also one of the downstream target proteins of ESR1. COH significantly decreased the expression of *Lif* mRNA, while YBSHD effectively increased its expression (Figure 5M), suggesting that YBSHD restored

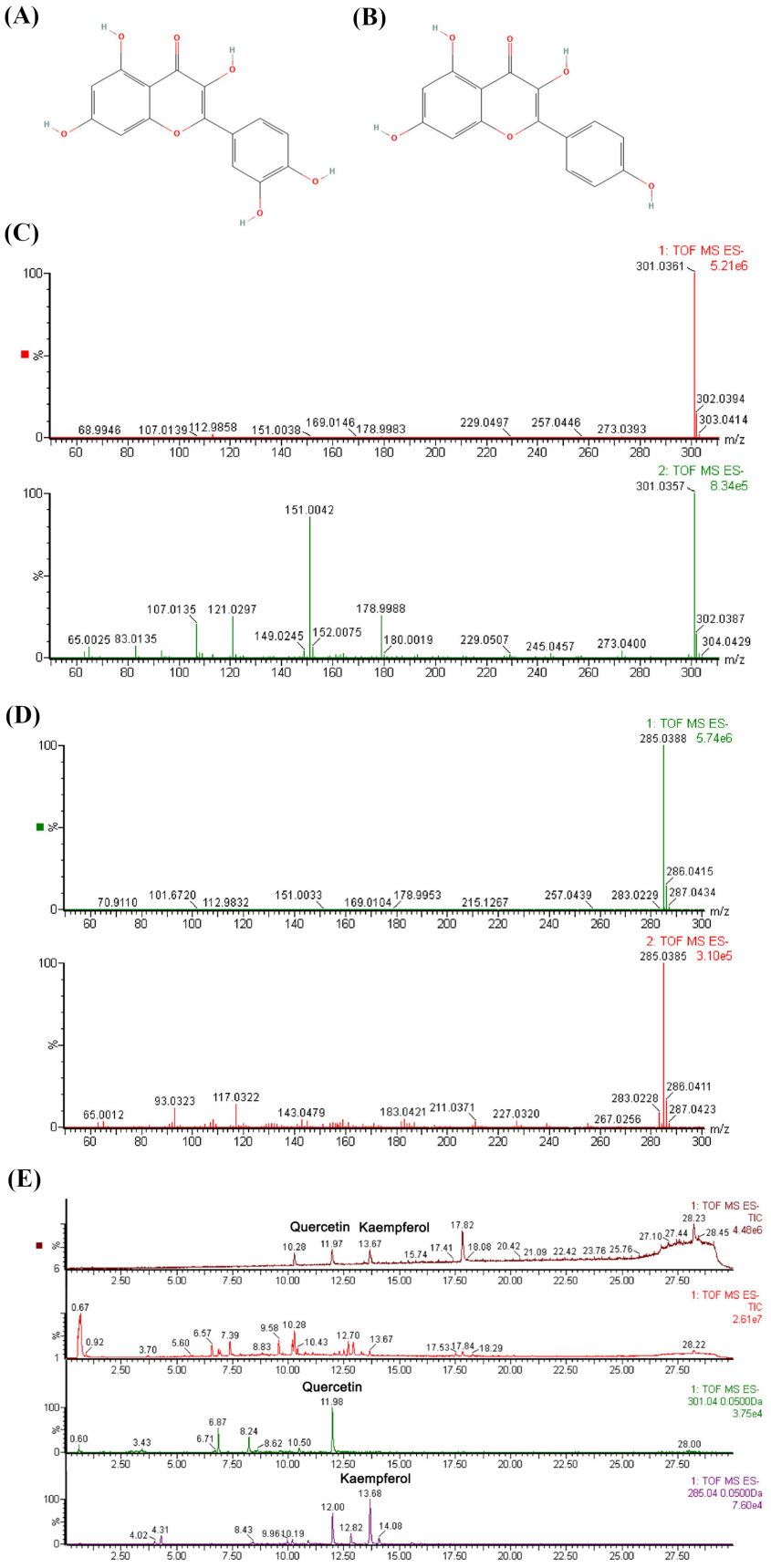

Figure 4. Fingerprint analysis of YBSHD using UPLC-UV/Q-TOF MS. Chemical structure of quercetin **(A)** and kaempferol **(B)**. Mass spectrum of the quercetin standard **(C)** and the kaempferol standard **(D)**. Primary and secondary mass spectrometry data are shown on the first and second lines, respectively. **(E)** Mass spectrum of YBSHD. The first line indicates the mass spectrum of the standards. The second line indicates the total ion chromatogram. Peaks for quercetin and kaempferol were detected in the third and fourth lines, respectively.

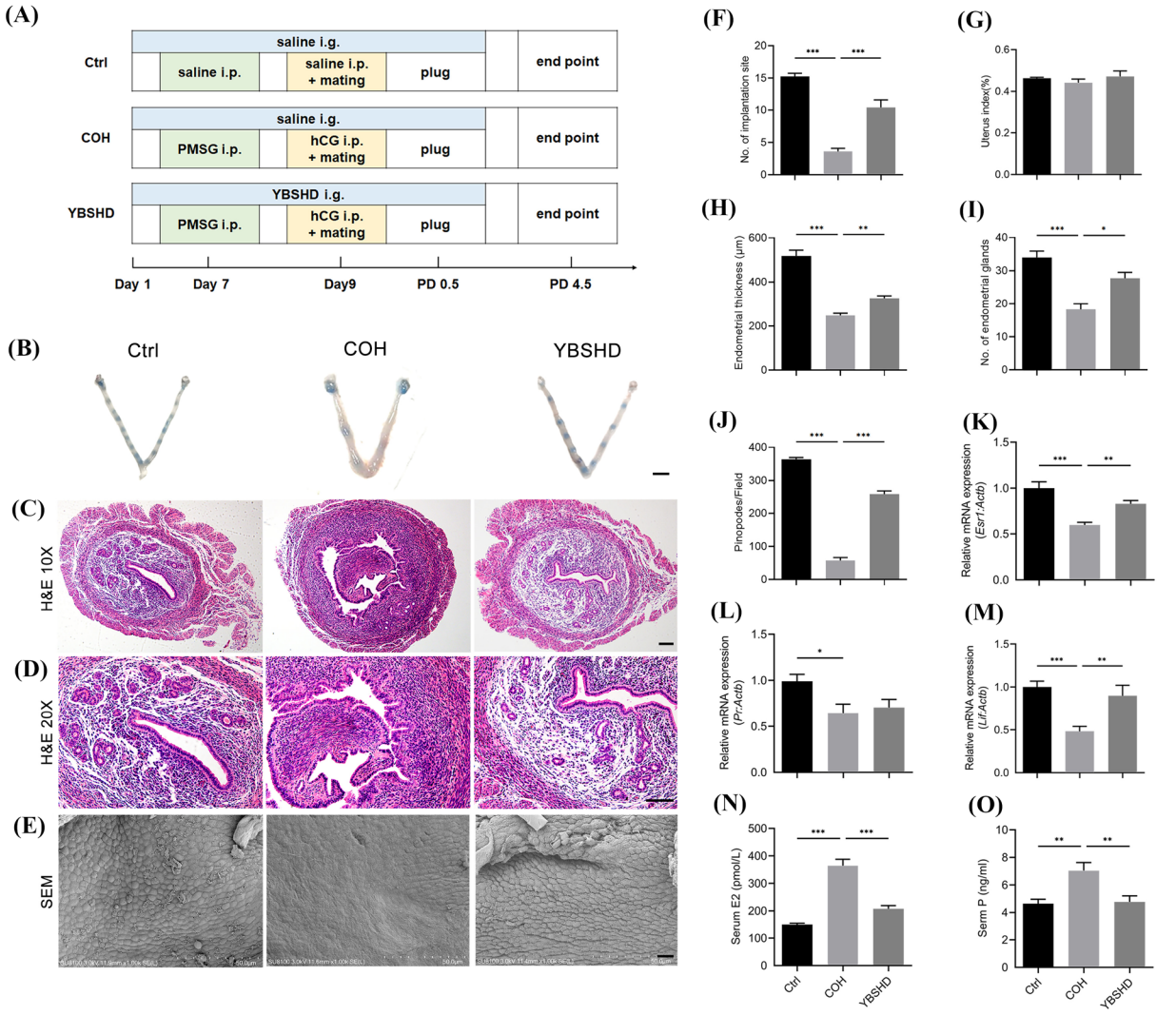

Figure 5. Improved implantation and endometrium were observed in PD 4.5 mice treated with YHSHD. (A) Schema of YBSHD treatment in a model involving COH mice. **(B)** Representative images of implantation sites dyed with Chicago sky blue in the PD 4.5 uterus among the three groups. **(C)** Representative H&E staining of endometrial thickness on PD 4.5 mice in individual groups (10 ×). **(D)** Representative images of H&E staining showed the changes in uterine glands and stroma in each group (20×). **(E)** Representative images of endometrial pinopodes according to SEM (1000×). Data depict the number of embryo implantation sites **(F)** and the uterine index **(G)** in mice treated as indicated. Quantitative results for uterine endometrial thickness **(H)** and glands **(I)** in PD 4.5 mice. **(J)** The average number of pinopodes per field in PD 4.5 mice. The expression of *Esr1* **(K)** and *Pr* **(L)** mRNA in the murine uterus was compared in different groups using PCR. **(M)** The expression of endometrial receptivity biomarker *Lif* was elevated by YBSHD compared to the level in COH mice. The effect of YBSHD on serum estrogen (E2) **(N)** and progesterone (P) **(O)** was assessed using ELISA. Data are expressed as the mean ± SEM. *$P < 0.05$ **$P < 0.01$, ***$P < 0.001$. Scale bar, **(B)** 500 μm, **(C)** 200 μm, **(D)** 50 μm, and **(E)** 100 μm.

endometrial receptivity.

3.9. Effect of YBSHD on murine estrogen and progesterone secretion in the COH model

During superovulation, exogenous gonadotropins not only promote follicular development but also change the hormone levels through a feedback regulation mechanism that affects the hypothalamic-pituitary-ovary axis (*16*). Therefore, the serum sex hormone levels in mice in each group were measured at PD 4.5. Results suggested that the average levels of serum estrogen were significantly elevated in the COH group compared to those in the Ctrl group. In contrast, YBSHD administration significantly down-regulated estrogen levels (Figure 5N). The serum levels of progesterone were also observed in different groups. The Ctrl group had an average of 4.64 ng/ml, while the COH group had an average of 7.04 ng/ml, which was 1.52 times higher than that in the Ctrl group. YBSHD significantly reduces the serum level of progesterone to the normal level (Figure 5O), indicating that it can ameliorate the supraphysiological level of the hormone caused by superovulation.

3.10. Effect of YBSHD on endometrial inflammation

Excessive hormones induced by COH may result in ovarian hyperstimulation syndrome (OHSS), which

is often followed by an inflammatory state (*17*). GO enrichment analysis implied that the role of YBSHD in endometrial receptivity may involve the regulation of hormones and inflammatory responses. Therefore, the GeneMANIA database was used to predict the key molecules that YBSHD regulates in the hormone-inflammatory network. As shown in Figure 6A, 76 genes were involved in the regulation of biological functions such as the response to steroid hormones, the regulation of hormone secretion, the response to peptide hormones, the regulation of inflammatory response, cell chemotaxis, and the production of molecular mediators in inflammatory responses. *Esr1*, but not *Pr*, was found to be down-regulated by YBSHD, pointing

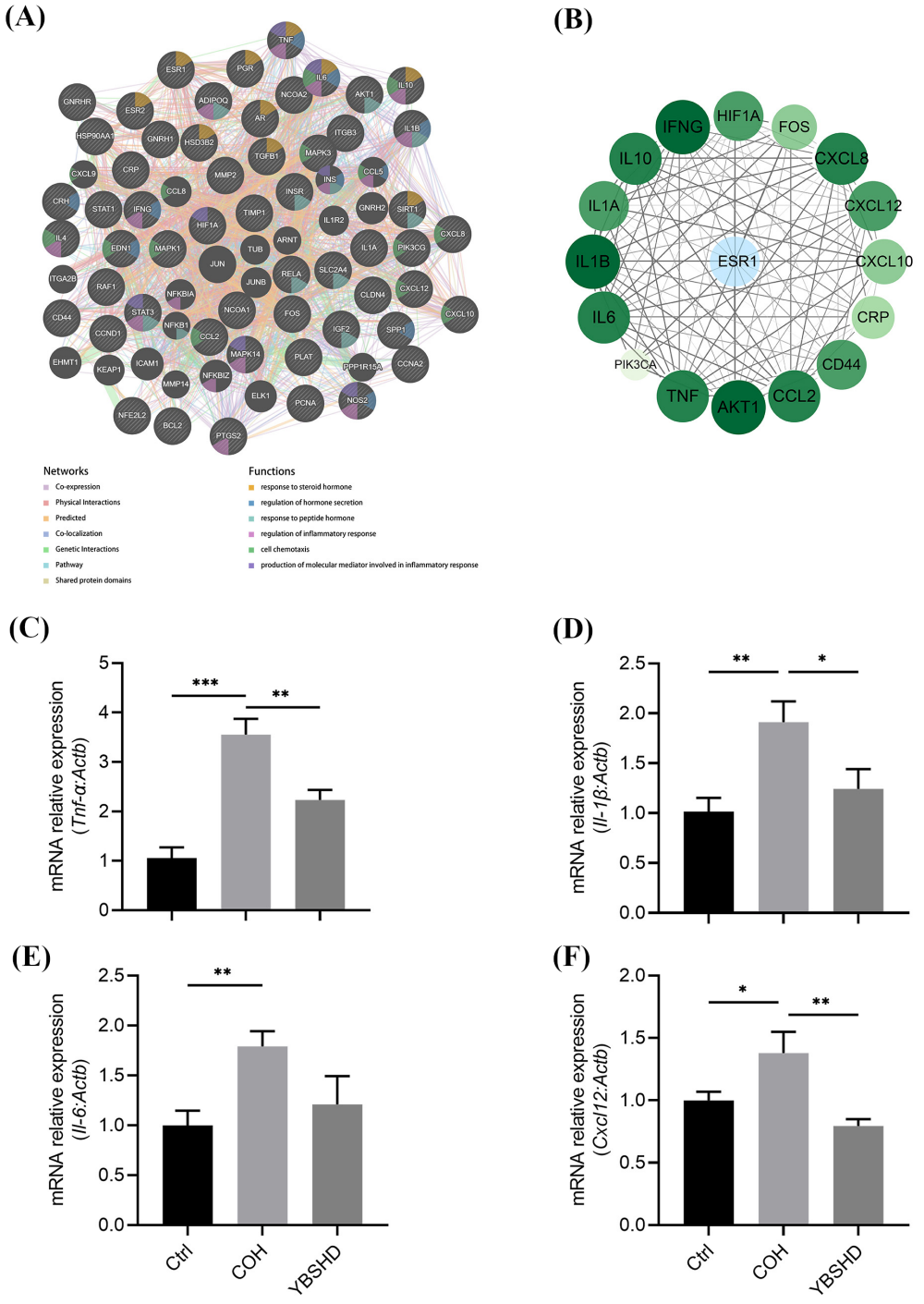

Figure 6. Regulation of YBSHD in the hormone-inflammatory network. (A) Network and function of YBSHD on hormone-inflammation regulation. **(B)** PPI analysis focused on the hormone-inflammatory network regulated by YBSHD. PCR revealed the expression of *Tnf-α* **(C)**, *Il-1β* **(D)**, *Il-6* **(E)**, and *Cxcl12* **(F)** mRNA in the murine uterus of different groups. Data are expressed as the mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

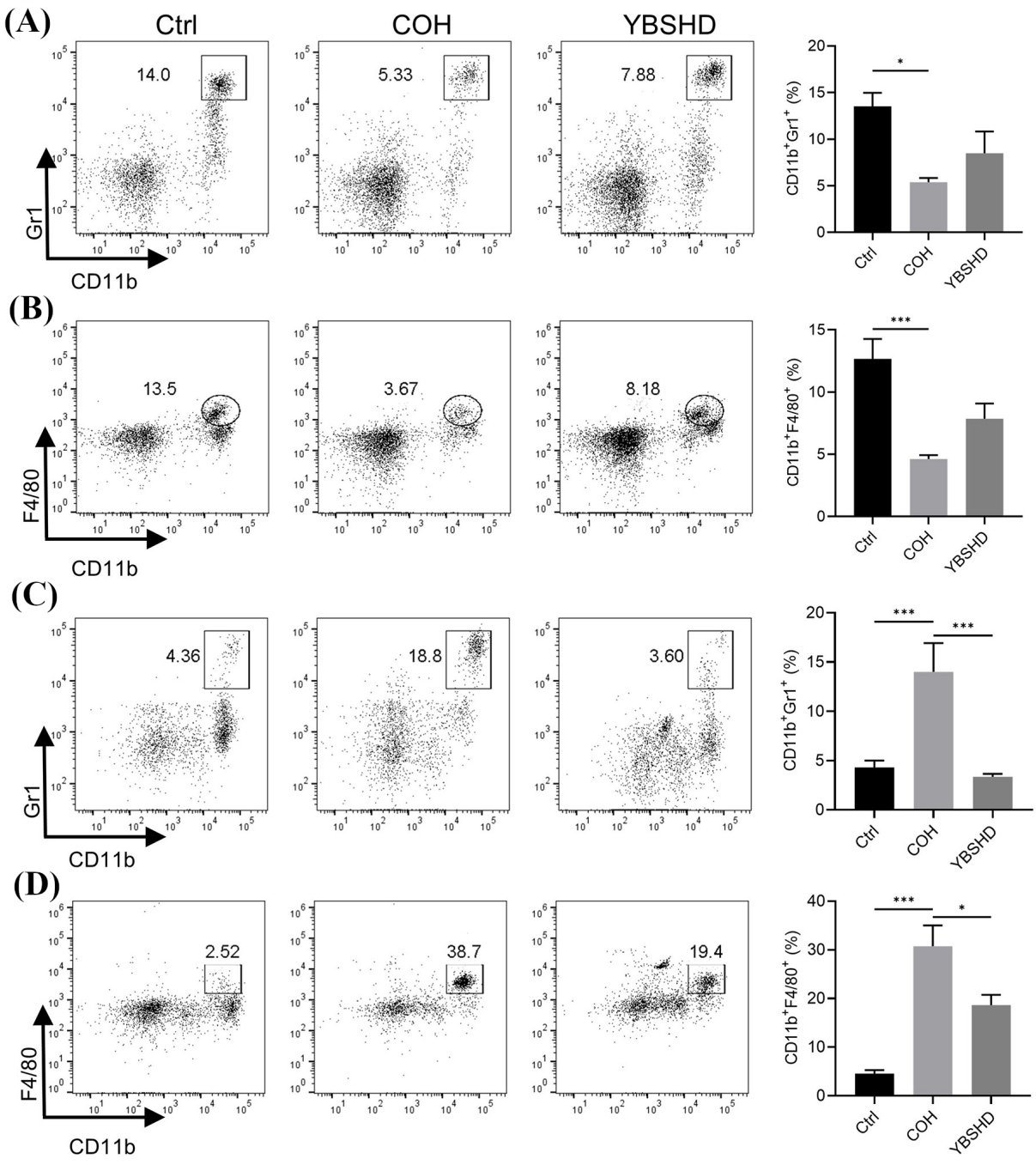

Figure 7. Flow cytometry analysis revealed changes in the uterine and blood immune cell population in COH mice treated with YBSHD. **(A)** The population of uterine $CD11b^+Gr\text{-}1^+$ neutrophils in mice. **(B)** The population of $CD11b^+F4/80^+$macrophages in the murine uterus was detected. **(C)** The population of $CD11b^+Gr\text{-}1^+$ neutrophils in the peripheral blood of mice. **(D)** The population of $CD11b^+F4/80^+$macrophage in the peripheral blood of mice. Data are expressed as the mean ± SEM. $^*P < 0.05$, $^{***}P < 0.001$.

towards the fact that *Esr1* and its downstream pathway regulate endometrial receptivity in COH mice after YBSHD treatment. GO enrichment analysis identified 17 key molecules, including ESR1 and inflammation-related genes (chemokines, pro-inflammatory and anti-inflammatory cytokines). The complex interaction between them was further depicted by PPI analysis (Figure 6B).

Given that COH may trigger an inflammatory response, the impact of YBSHD on local inflammatory mediators was further investigated. Results indicated that the expression of *Tnf-α* and *Il-1β* mRNA in the murine uterus of the COH group increased significantly. YBSHD significantly reduces the expression of *Tnf-α* and *Il-1β* (Figure 6C-D). A similar trend was seen in the expression of *Il-6*, but the difference was not significant (Figure 6E). The migration and interaction of immune cells during inflammation is known to be mediated by chemokines. The expression of the chemokine *Cxcl12* was found to be significantly higher in the COH group,

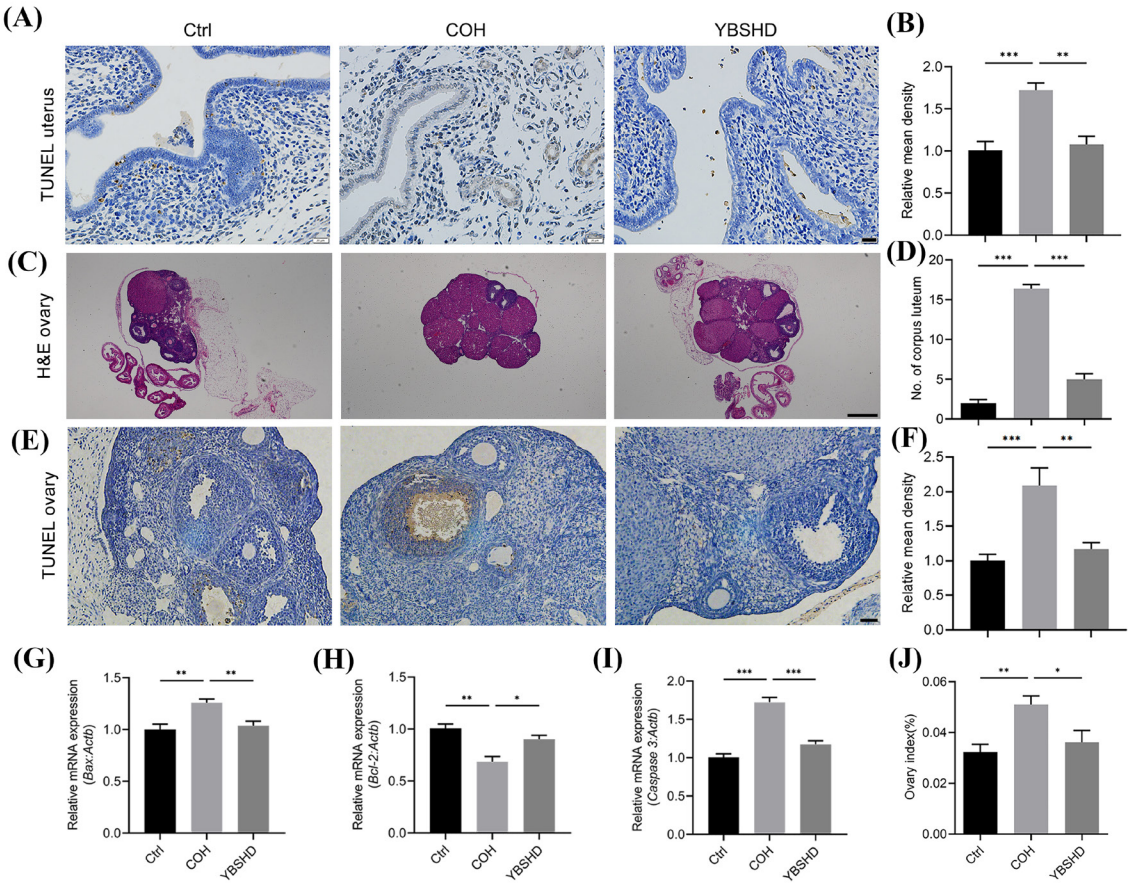

Figure 8. YBSHD affects apoptosis in the ovaries and uterus of COH mice. (A) TUNEL staining showed apoptosis in the murine uterus of different groups. **(C)** Morphology of a murine ovary characterized by H&E staining. **(D)** The number of corpora lutea in COH mice were compared after administration of YBSHD. **(E)** The effect of YBSHD on ovarian apoptosis is indicated by TUNEL staining. The quantified relative mean density of TUNEL staining in the ovaries **(B)** and uterus **(F)** of three groups. qPCR testing detected mRNA expression of the apoptosis molecules *Bax* **(G)**, *Bcl-2*(**H**), and *Caspase 3* **(I)**, regulated by YBSHD. **(J)** The ovary index was calculated in all mice of the groups. Data are expressed as the mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Scale bar, **(A)** 20 μm, **(C)** 500 μm, and **(E)** 50 μm.

and YBSHD significantly lowered its level (Figure 6F). As a result, YBSHD may ameliorate the inflammatory state and uterine immune environment through the regulation of inflammatory factors and chemokines.

Flow cytometry was performed to compare the myeloid cells between uterine and peripheral cells in PD 4.5 mice. Both the proportion of $CD11b^{+}Gr1^{+}$ neutrophils and $CD11b^{+}F4/80^{+}$ macrophages in peripheral blood significantly decreased in COH mice compared to Ctrl mice, while this was reversed by YBSHD (Figure 7A-B). In contrast, the proportion of $CD11b^{+}Gr1^{+}$ neutrophils and $CD11b^{+}F4/80^{+}$ macrophages from the murine uterus significantly increased in COH mice (Figure 7C-D). The opposite change in uterine and peripheral cells implied that peripheral neutrophils and macrophages may migrate to the uterus in response to inflammation. YBSHD treatment reversed the changes in the proportion of these two types of cells in the uterus and peripheral blood, possibly by downregulating the expression of the chemokine *Cxcl12* to inhibit migration from the peripheral blood to the uterus.

3.11. Effect of YBSHD on uterine and ovarian apoptosis

Apoptosis exists in embryo attachment and infiltration, while supra-physiological estrogen and the ensuing inflammation caused by COH may result in excessive apoptosis in the uterus, thereby compromising embryo implantation. KEGG and GO enrichment analysis indicated that YBSHD might regulate endometrial receptivity through modulation of apoptotic signaling pathways. The level of apoptosis in the murine uterus was subsequently quantified in all groups. Quantitative TUNEL assays revealed extensive apoptosis in both the luminal and glandular epithelium of the murine uterus induced by COH (Figure 8A-B), demonstrating that COH elicited marked endometrial apoptotic responses. The decrease in TUNEL-positive cells in endometrium treated with YBSHD suggested that this herbal formulation effectively suppresses COH-induced apoptosis.

Given the established impact of exogenous gonadotropins on mitochondrial dysfunction in granulosa and endometrial cells (*18*), key regulators of

the mitochondrial apoptotic pathway were examined in murine uterine tissues. PCR revealed significantly elevated expression of the pro-apoptotic Bcl-2 family member *Bax* in uterine tissues subjected to COH, and this was markedly attenuated by YBSHD (Figure 8G). Measurement of anti-apoptotic *Bcl-2* mRNA revealed significantly reduced expression in the COH group versus controls, while YBSHD treatment markedly restored its expression (Figure 8H). Significantly elevated levels of *Caspase 3* mRNA, a critical executor of apoptosis, were evident in the COH group. YBSHD administration significantly attenuated *Caspase 3* expression (Figure 8I), implying its protective role against COH-induced uterine apoptosis.

Considering the potential disruption that COH may cause in the hypothalamic-pituitary-ovarian axis, ovarian morphology in PD 4.5 mice was histologically evaluated (Figure 8C). COH induced pronounced ovarian enlargement (evidenced by elevated ovarian index) alongside follicular depletion, increased atretic follicles, and a ~3-fold accumulation of corpora lutea, which aligns with the expected ovarian hyperstimulation (Figure 8D&J). YBSHD treatment restored ovarian volume, lowered the ovarian index, and reduced excessive corpora lutea, confirming its protective role in ovarian hyperstimulation. TUNEL staining was also performed to assess YBSHD's impact on ovarian apoptosis in all groups (Figure 8E). Ovarian tissues in the Ctrl group exhibited virtually no TUNEL-positive cells. COH induced prominent TUNEL-positive signals in follicles, whereas YBSHD administration significantly reduced the relative mean density of TUNEL-positive cells (Figure 8F), indicating reduced ovarian apoptosis.

4. Discussion

In recent years, about 1/7 of couples worldwide have been affected by infertility (*19*), while UI accounts for about 30% to 60.3% (*20,21*). COH, a widely accepted recommendation in guidelines for the treatment of UI (*22,23*), can induce a supraphysiological increase in maternal estrogen levels. It may lead to the dislocation or deviation of the embryo and endometrial window period, thereby compromising embryo implantation and the pregnancy rate (*24,25*). Therefore, understanding the potential negative effects of COH on the endometrium and optimizing treatment strategies has become the focus of research to improve pregnancy outcomes. Through our inclusion and exclusion criteria, patients with UI undergoing COH were enrolled as a comparatively standardized baseline population for evaluation of endometrial receptivity. Improvement of the live birth rate in the YBSHD group suggests a potential benefit of YBSHD for patients with UI undergoing COH.

In recent years, network pharmacology has been used as a preliminary approach to reveal the potential mechanism of the active ingredients in TCM, and molecular docking is used to predict the binding affinity between the drug components and receptors (*26*). Network pharmacology and enrichment analysis indicated that YBSHD contains a variety of potential effective ingredients and may improve endometrial receptivity by regulating hormone response, inflammation, apoptosis, *etc*. PPI indicated that some molecules related to regulating hormone response, inflammation, and apoptosis were located in the core position, including ESR1, IL1B, IL6, TNF, and BCL2. UPLC-UV/Q-TOF MS technology indicated two active components of YBSHD, quercetin and kaempferol, which confirmed the predictions of network pharmacology. Molecular docking further confirmed the binding affinities of quercetin and kaempferol to TNF, ESR1, BCL2, IL1B, and IL6, implying a favorable binding affinity between the core components of YBSHD and targets of endometrial receptivity. Moreover, quercetin inhibited the inflammation and apoptosis in decidual cells stimulated with lipopolysaccharide (*27*), and it improved endometrial receptivity in diabetic mice (*28*). Kaempferol alleviated uterine and ovarian apoptosis in cypermethrin-exposed rats (*29*) and had anti-inflammatory action in a preeclampsia rat model (*30*). Quercetin and kaempferol increase ESR1 expression in rats with diminished ovarian reserve, and strong binding between these two compounds and ESR1 has been confirmed by surface plasmon resonance analysis (*31*). In line with these studies, our research demonstrated that YBSHD inhibits inflammation and cell apoptosis and it improves endometrial receptivity in COH mice. Thus, we hypothesize that the relative mechanisms are mediated by the regulation of ESR1 *via* quercetin and kaempferol. The role and mechanism of quercetin and kaempferol in endometrial receptivity will be investigated in a future study.

Previous animal experiments revealed that ovariectomized rats supplemented with high-dose estrogen had hydrops in the abdominal cavity and uterine horns, increased organ wet weight, and intestinal loop expansion (*32*). In the current study, YBSHD effectively alleviated the index and volume of murine ovaries elevated by COH. In addition, COH leads to estrogen secretion reaching its peak in the early luteal phase, which should have occurred in the mid-luteal phase. It causes a deviation in the window of implantation, which in turn affects the expression patterns of endometrium-related genes and proteins (*33,34*). The window of uterine receptivity opens only at lower estrogen levels but not at higher levels (*25,35*), which might explain the better embryo implantation in the YBSHD group as verified with Chicago sky blue dye, H&E staining, and electron microscopy, where no supraphysiological estrogen exposure occurred.

Ovarian stimulation can result in a reduction in ESR1 compared to natural cycles (*36,37*). In the current study, network pharmacology indicated that the reduction in

COH-induced supraphysiological estrogen by YBSHD may be mediated through ESR1. ESR1 is a key factor in the response of the endometrium to estrogen. In the early stages of pregnancy, estrogen activates the downstream signal network through ESR1, regulates the proliferation and differentiation of endometrial epithelial cells, and creates a suitable microenvironment for embryo implantation. The current study showed that YBSHD effectively up-regulates ESR1, activates the downstream target protein LIF (one of the biomarkers of endometrial receptivity), and inhibits excessive hormone levels, providing a stable endocrine environment for smooth embryo implantation.

Studies have found that patients with OHSS appear to be in an inflammatory process, accompanied by high levels of IL-1β, TNF-α, and IL-6 and high serum estrogen (*17*). Long-term estrogen supplementation can induce an M1-type inflammatory phenotype in mouse macrophages and promote the secretion of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 (*38*). Changes in inflammation-related molecules may accelerate the formation of a pro-inflammatory environment, leading to a disorder of the endometrial environment and affecting the failure of embryo implantation. Network pharmacology indicated a hormone-inflammatory interaction in the regulation of endometrial receptivity by YBSHD. We therefore analyzed the expression of genes related to inflammation in the mouse uterus and found that YBSHD significantly suppressed the expression of *Tnfα*, *Il1β,* and *Cxcl12* while slightly downregulating *Il6*. These findings substantiate the anti-inflammatory effect of YBSHD in COH mice.

Immune cells are essential for endometrial receptivity and embryo implantation. Increased M1-type macrophages have been found in the endometrium of patients with repeated implantation failure during the window of implantation, along with elevated proportions and cytotoxicity of uterine natural killer cells (*39*). An imbalance in macrophage polarization may disrupt the maternal-fetal interface during early pregnancy, fostering a pro-inflammatory microenvironment that compromises embryo implantation and pregnancy maintenance (*40*). Therefore, flow cytometry was performed to analyze the proportion of and phenotypic changes in immune cells in the murine uterus from each group. Results revealed that COH mice had an increased proportion of neutrophils ($CD45^+CD11b^+Gr1^+$) and macrophages ($CD45^+CD11b^+F4/80^+$) in the endometrium, indicating a disrupted endometrial immune microenvironment. Interestingly, peripheral blood indicated a decreased proportion of both neutrophils and macrophages in COH mice, suggesting an inverse correlation with their endometrial counterparts. We therefore speculate that some endometrial immune cells may have migrated from the peripheral circulation. A pro-inflammatory chemokine, CXCL12 binds to its receptor CXCR4 and recruits T lymphocytes and monocytes/macrophages that express the receptor to inflammatory sites, playing a classic chemokine role in immune responses (*41*). Elevated expression of *Cxcl12* was noted in the COH group, suggesting its potential involvement in the chemotaxis of peripheral immune cells. YBSHD significantly inhibited the expression of *Cxcl12* in the uterus and reduced the proportion of neutrophils and macrophages in the endometrium, indicating that YBSHD may improve the uterine immune microenvironment by regulating the local inflammatory response and immune cell subsets, which are conducive to embryo implantation.

A pro-inflammatory cytokine, TNF-α promotes apoptosis and participates in two patterns of cell death: apoptosis and necroptosis (*42,43*). TNF-α can induce apoptosis of follicular granulosa cells and endometrial epithelial cells during follicular atresia (*44,45*). We previously noted changes in the *Tnf-α* expression regulated by YBSHD, and network pharmacology suggested that YBSHD may affect the apoptotic pathway. Therefore, we further examined apoptosis in the murine uterus and ovary. TUNEL staining indicated an increase in the apoptosis of uterine and ovarian tissues as well as an increased number of ovarian atretic follicles in COH mice. The extent of apoptosis in the uterus and ovary decreased in the YBSHD group, and expression of the pro-apoptotic molecule *Bax* and the downstream execution molecule *Caspase 3* was downregulated, while expression of the anti-apoptotic molecule *Bcl2* was upregulated. These results indicate that YBSHD may not only alleviate the inflammatory response caused by COH but also reduce cell apoptosis.

The current study has shown that YBSHD alleviates the reduced endometrial receptivity caused by COH through the mechanism of 'anti-inflammatory-anti-apoptotic-hormone regulation', which provides new insight into improving pregnancy outcomes and optimizing the COH regimen. However, this study has several limitations. While patients with UI were selected to minimize confounders related to endometrial receptivity, the heterogeneity of UI should be considered. In addition, there may have been possible selection bias in the retrospective study. Although a COH mouse model was used to match the clinical scenario of patients with UI undergoing COH, murine studies cannot fully simulate the dynamic changes in human tissue. The mechanism by which YBSHD improves endometrial receptivity and regulates ESR1, LIF, and its downstream signaling pathways has not been fully explored. In the future, its efficacy needs to be verified through multi-center randomized trials and the synergistic effect and molecular mechanism of specific components of YBSHD need to be analyzed, addressing the limitations of COH therapy and opening up a new avenue for integrated traditional Chinese and Western medicine to bring about successful pregnancy outcomes in patients with UI.

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Original Article

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Hearing impairment in Parkinson's disease models: Possible relation with changes in cochlear efferent fibers

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SUMMARY: Hearing impairments, as a prevalent and debilitating non-motor symptom of Parkinson's disease (PD), remain unclear in mechanisms. In this work, we established PD mouse and rat models by using 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA), respectively, and investigated their hearing functions and potential mechanisms through auditory brainstem response (ABR), distortion product otoacoustic Emissions (DPOAE), noise exposure, immunofluorescence labeling, volumetric measurement, and colocalization analysis. In MPTP-induced PD mice, we observed significant cholinergic fibers decompensation, heterogeneous dopaminergic fibers damage of cochlear efferent fibers, and adrenergic sympathetic fibers marked loss in the osseous spiral lamina (OSL), corresponding to insignificant cochlear hair cells, ribbon synapse alteration, and auditory sensitivity injury. While in 6-OHDA-induced PD rats, asymmetric alterations in cochlear cholinergic, dopaminergic fibers were found, accompanied by inconsistent adrenergic changes in the OSL, which matched unilateral hair cells, ribbon synapse damage, and hearing loss. Overall, findings from this work indicate that pathological alterations in the cochlea of PD mice and rats, particularly in efferent fibers, may be closely relevant to peripheral hearing alterations.

Keywords: Parkinson's disease, hearing loss, cholinergic fibers, dopaminergic fibers, sympathetic fibers

1. Introduction

Parkinson's disease (PD), the second most common progressive neurodegenerative disorder, is a major contributor to rising global disability rates. In recent years, PD-related prevalence, disability rates, and mortality have shown an upward trend (*1*). The etiology of PD remains unknown. It is characterized primarily by early dopaminergic neuronal loss in the substantia nigra of the basal ganglia, accompanied by classic Parkinson's disease manifestations and secondary motor symptoms. Furthermore, non-motor symptoms that usually precede typical PD manifestations have garnered significant attention, which include fatigue, gastrointestinal disturbances, and sensory deficits (visual impairment, olfactory dysfunction, and hearing impairment). Compared with studies on olfactory dysfunction and visual impairment, previous research on hearing impairment has been relatively insufficient (*2*). Population-based case-control studies indicate a 1.6% association between clinical hearing impairment and PD (*3,4*). Interestingly, studies have found that, similar to motor symptoms, auditory symptoms in PD patients also exhibit lateralization — meaning more pronounced hearing loss occurs on the side with more severe PD symptoms (*5*). However, the mechanisms underlying hearing impairment in PD patients remain unclear. Recent research suggests its potential association with dysfunction of dopamine transporters in the basal ganglia (*6*).

Dopamine is primarily produced in the striatal structures of the substantia nigra and regulates processes such as movement, attention, reward, and motivation. Growing evidence indicates dopamine also participates in auditory processing, with fibers present in auditory structures including the auditory cortex, thalamus, superior olivary complex, and inferior colliculus. This involvement occurs primarily through dopamine receptors, notably dopamine receptor 2 (D2) (*7,8*). Recent studies have identified the subperiventricular nucleus (SPF) of the thalamic bundle as a potential key hub coordinating the balance of neurotransmitters — including glutamate and glycine — between various auditory structures such as the inferior colliculus and superior olivary complex (*7,9*). Dopaminergic fibers in the cochlea primarily originate from lateral olivocochlear

neurons (LOC), projecting onto afferent fibers adjacent to inner hair cells. These fibers inhibit excessive excitation of afferent neurons *via* nearby dopamine receptor 1 (D1) and D2 receptors, predominantly D2 receptors, acting in concert with cholinergic fibers — another component of LOC fibers — to coordinate function (*7,10,11*). However, the precise function of LOC remains unclear. The prevailing view suggests it possibly majors in adaptation to acoustic environments and balances subtle bilateral activity. In contrast, medial olivocochlear neurons (MOC) primarily project cholinergic fibers that synapse with the basal ends of outer hair cells. They mainly regulate excitability of bilateral outer hair cells, participating in auditory sensitivity modulation under varying sound backgrounds and spatial auditory discrimination (*10,12,13*).

Recent research on the auditory system in PD has primarily focused on regions such as the auditory cortex and basal ganglia, with greater emphasis on its role in auditory-related cognition and emotion (*10,14,15*). However, studies on peripheral hearing, particularly cochlear pathophysiological changes in PD models, remain scarce. Thus, this study mainly focuses on the hearing changes and cochlear pathological alterations, especially changes in olivocochlear efferent fibers, in PD mice and rat models, to provide new insights for investigating mechanisms and treatments for hearing loss in PD.

2. Materials and Methods

2.1. Animals and drug treatments

Due to the hearing-protective effects of estrogen and the high mortality rate of female mice in PD modeling, only male animals were used in this study (*16,17*). Male C57BL/6J mice (7 weeks old) and male Sprague-Dawley (SD) rats (10 weeks old) were purchased from the Animal Experiment Center of Peking University People's Hospital (PKUPH). This study was approved by the Institutional Animal Care and Use Committee (IACUC) at PKUPH (No. 2023PHE025). All animals were housed in a controlled, specific pathogen-free environment (temperature, 23 ± 3°C; humidity, 55% ± 15%; 12/12 h light/dark cycle) with free access to food and water. Following work based on previous studies, mice with abnormal hearing were excluded (*18-22*). The remaining mice were randomly assigned to two groups: the MPTP group (model group), which received intraperitoneal injections of MPTP-HCl (Sigma, St. Louis, MO, USA, M0896, 30 mg/kg) once daily for 5 days, while the control group received an equivalent volume of saline *via* intraperitoneal injection once daily for 5 days (*18-22*). Subsequent experiments were conducted according to the experimental protocol without a time interval. Mice in two groups underwent behavioral testing, open field test, and audiological assessment, ABR and DPOAE, followed by tissue collection for pathological examination of the brain and cochlea immediately. Based on the successful establishment of the PD model, confirmed by behavioral and pathological analyses, findings regarding audiology and cochlear morphology were obtained (Supplementary Figure S1 A, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*). SD rats were screened for hearing abnormalities and excluded as described previously (*23-26*). Rats underwent unilateral 6-OHDA lesions of the substantia nigra pars compacta (SNc). Briefly, rats anesthetized with sodium pentobarbital (40 mg/kg, ip) were fixed in a stereotaxic apparatus (SN-2 N, Narishige, Tokyo, Japan) and injected with 6-OHDA (2 μg/μL) into the right SNc (AP -5.2 mm, ML -2.0 mm, DV -8.0 mm). Fifteen minutes before 6-OHDA injection, rats received pretreatment with dexepamine (25 mg/kg, ip) to protect noradrenergic neurons. Control rats received an equivalent injection of physiological saline containing 0.02% ascorbic acid (*23-26*). Subsequent procedures were performed according to the experimental protocol after 3 weeks. Both groups of rats underwent behavioral testing, open field test, and audiological assessment, ABR and DPOAE, followed by tissue collection for pathological examination of the brain and cochlea immediately. Based on successful establishment of the PD model, confirmed by behavioral and pathological analyses, findings regarding audiology and cochlear morphology were obtained (Supplementary Figure S2 A, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*).

2.2. Open field test

Before testing, mice or rats were acclimated for 2 hours in their home cages within a quiet room. The open field arena (mice: 50 cm × 50 cm × 40 cm, white background; rats: 4 × 50 cm × 50 cm × 40 cm, white background) was placed in a soundproof chamber illuminated by indirect artificial light. The arena was thoroughly cleaned with a 5% ethanol/water solution between trials. One animal (mouse or rat) was positioned in the center of the arena, and spontaneous behavior was recorded for 10 minutes. Subsequently, video recordings were evaluated using the SMART video tracking system (Panlab, Barcelona, Spain).

2.3. Brain tissue immunofluorescence and immunohistochemistry

Mice or rats were deeply anesthetized and perfused with physiological saline, followed by injection of 4% polyformaldehyde (PFA) in 0.1 mol/L phosphate-buffered saline (water, PBS, pH 7.4). Brains were dissected and immersed in the same fixative for 12 hours, then dehydrated in 30% sucrose solution. Frozen brain tissue was sectioned into 30 μm-thick slices using a cryostat (Leica Biosystems, Nuremberg, Germany).

For immunofluorescence, sections were rinsed with PBS and blocked with a PBS solution containing 5% BSA and 0.5% Triton X-100 as antibody diluent. Sections were incubated with anti-tyrosine hydroxylase antibody (1:100, 58844, Cell Signaling Technology) at 4°C for 12 hours, washed three times with PBS, and subsequently incubated with secondary antibody for 2 hours. Finally, slides were sealed with anti-fade mounting medium containing DAPI (P0126, Beyotime, Shanghai, China) and imaged using a confocal microscope (Leica, Stellaris). For immunohistochemistry, sections were blocked with PBS containing 5% BSA, 1% normal goat serum, and 0.3% Triton X-100, then incubated overnight at 4°C with anti-tyrosine hydroxylase antibody (1:300, 58844, Cell Signaling Technology). Sections were incubated at room temperature for 2 h with biotinylated secondary antibody, followed by incubation for 1 h with ABC reagent (1:500, Vector Laboratories) and visualized using DAB substrate (Vector SK-4100). Slides were dehydrated through graded ethanol, cleared in xylene, and mounted with neutral resin coverslips. Brightfield images were captured using an Olympus BX53 microscope (Olympus).

2.4. ABR and DPOAE measurement

Before ABR testing, mice or rats were anesthetized *via* intraperitoneal (i.p.) injection of 0.7% sodium pentobarbital (25–50 mg/kg). For ABR recordings, three needle electrodes were placed subcutaneously: at the vertex, behind the test ear, and on the contralateral auricle. ABR thresholds were measured and recorded using a TDT system (RZ6 TDT; Tucker Davis Technologies hardware and SigGen/BioSig software; Alachua, Florida, USA) at a sampling rate of 21.1 Hz, delivered *via* a closed-field microphone system at various stimulus frequencies (4, 8, 16, 24, and 32 kHz). Sound stimuli began at 90 dB SPL and were decreased in 5 dB increments until ABR waves were no longer readily detectable. DPOAE measurements were conducted following ABR testing. The f1-f2 DPOAE was evaluated using TDT's real-time signal processing system II (RZ6 TDT; Tucker Davis Technologies hardware and SigGen/BioSig software). The DPOAE threshold is defined as the peak at 2f2-f1. During the testing process, a double-blind approach was adopted, and the sample size was determined based on biological replicates.

2.5. Noise exposure

Mice were placed in stainless steel cages positioned beneath a loudspeaker. Noise was generated by a loudspeaker (Aijie Audio Equipment Factory) driven by a power amplifier (MF-1201 MOSTET, ATech) and attenuator (PA5 TDT, Alachua, FL, USA). White noise at 108 dB sound pressure level (SPL) was used for 2 h to induce exposure. Calibration was measured using a sound level meter (Model 1200; Quest Technologies) before exposure. Ambient background noise around the cages was 45 dB. Control mice were placed in the same cages and the noise exposure chamber without noise activation for 2 h.

2.6. Immunofluorescence of the basilar membrane

Mice or rats were euthanized using carbon dioxide anesthesia, and bilateral cochleae were collected and fixed overnight in 4% paraformaldehyde at 4°C. The following day, cochleae were decalcified at room temperature using 10% EDTA for 5–6 hours (for mice) or 48–72 hours (for rats). After decalcification, the basilar membrane was carefully dissected and placed in PBS containing 0.3% Triton X-100 for 10 minutes; this process was repeated twice. Subsequently, the basilar membrane was blocked with 10% donkey serum for 2 hours, then incubated with the primary antibody overnight at 4°C. The following day, the basilar membrane was washed three times with PBS for 10 minutes each, and then incubated with the secondary antibody for 1 hour in the dark. Finally, the basilar membrane was stained with or without DAPI for 15 minutes. After washing as described above, the sample was mounted in medium and imaged using a confocal microscope (Leica, Stellaris).

Hair cells counting: The basilar membrane of the cochlea was divided into three turns, labeled with phalloidin (1:2000, A30104, ThermoFisher), and quantified under a 40× objective field.

Synaptic ribbon counting: Synaptic ribbons, associated with inner hair cells and myelinated afferent auditory nerves, were labeled with anti-C-terminal binding protein 2 (Ctbp2) (1:200, 612044, BD Biosciences), while hair cells were labeled with anti-Myosin 7a (1:500, 25–6790, Proteus-biosciences) and quantified under a 63× objective field.

Choline acetyltransferase (ChAT) and tyrosine hydroxylase (TH) quantification and localization: The basilar membrane of the cochlea was divided into three turns, labeled with ChAT (1:500, AB144P, Millipore), TH (1:100, 58844, Cell Signaling Technology), and phalloidin (1:2000, A30104, ThermoFisher). Images were observed in a 40×objective field and analyzed using ImageJ for volume measurement and colocalization analysis.

2.7. Statistical analysis

Data analysis was conducted using Microsoft Excel and GraphPad Prism version 8. Detailed statistical information is provided in the results section, figures, and figure legends. All data are presented as mean ± standard error of the mean (SEM). Statistical significance was determined using two-tailed unpaired *t* tests and two-way ANOVA followed by Sidak's multiple

comparisons test, as indicated in each figure. The exact values of *n* are provided where appropriate. *, **, ***, **** indicate $p < 0.05$, 0.01, 0.001, and 0.0001, respectively.

3. Results

3.1. No significant differences were observed in hearing and corresponding morphology between PD and control mice

The MPTP mouse model is widely used in PD pathogenesis (*18-22*). We established a PD mouse model according to the experimental protocol in Supplementary Figure S1 A, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*, and verified by behavioral and morphological assessments. In detail, mice with abnormal hearing were excluded, and remaining mice were randomly assigned to two groups: the MPTP group (model group), which received intraperitoneal injections of MPTP-HCl once daily for 5 days, while the control group received an equivalent volume of saline *via* intraperitoneal injection once daily for 5 days (*18-22*). Subsequent experiments were conducted according to the experimental protocol without a time interval. Mice in two groups underwent behavioral testing, open field test, and audiological assessment, ABR and DPOAE, followed by tissue collection for pathological examination of the brain and cochlea immediately. Based on the successful establishment of the PD model, confirmed by behavioral and pathological analyses, findings regarding audiology and cochlear morphology were obtained (Supplementary Figure S1 A, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*). As shown in Supplementary Figure S1 B-C (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*), MPTP-treated mice exhibited significantly impaired locomotor activity. Immunofluorescence and immunohistochemistry both revealed a significant reduction in dopaminergic neurons in the substantia nigra of PD mice compared to controls (Supplementary Figure S1 D-E, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*). Therefore, we confirmed the successful establishment of the PD mouse model. To assess hearing differences between control and PD mice, we performed ABR and DPOAE testing. As shown in Figure 1A and B, ABR and DPOAE thresholds did not differ significantly between groups. Notably, compared to the control group, the PD group exhibited a mild increase in DPOAE threshold at 32 kHz, although it was not significant. Next, we focused on the latency and amplitude of ABR wave I (Figure 1 C-D). No significant change in wave I latency was found between groups, but amplitude was lower in the PD group, though this difference was not significant. Notably, compared with other frequencies, the wave I amplitude gap between the PD and control group was much smaller at 32 kHz. Next, we labeled hair cells with phalloidin to assess the impact of modeling on hair cell survival (Figure 1 E-F). Results showed no significant difference in inner and outer hair cell survival between groups (Figure 1 G-H). And ribbon synapse labeled by Ctbp2 shown in Figure 2 I-J also exhibited no dramatic numbers change at all turns between groups, although in the middle and base turn, PD mice exhibited a mild reduction in synapse numbers with no significance. Interestingly, compared with regular distribution in the basal turn, synapses in the apical and medial regions seemed more disorganized.

3.2. $ChAT^+$ fibers in the PD mice cochlea exhibited post-injury compensatory changes

Then we labeled ChAT in hair cells. The results showed that $ChAT^+$ regions exhibited increased total fluorescence intensity, enlarged volume, and heightened average fluorescence intensity across the entire cochlear hair cell (Figure 2 D-F). Furthermore, $ChAT^+$ hair cell segments exhibited more pronounced increases in total fluorescence intensity and volume at the mid-basal transition region, while the mean fluorescence intensity of the $ChAT^+$ hair cell fraction showed a more pronounced increase in the apical-medial turn. Next, we performed quantitative analysis of the $ChAT^+$ portion in inner hair cells. We found that the total fluorescence intensity, total volume, and average fluorescence intensity of the inner hair cell portion also increased. The total fluorescence intensity and volume of the $ChAT^+$ inner hair cell portion in the apical and basal turns appeared to increase more significantly. At the level of individual inner hair cells (Supplementary Figure S3 A-C, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*), $ChAT^+$ total fluorescence intensity, total volume, and average fluorescence intensity changes were similar to the entire $ChAT^+$ inner hair cell segment. Subsequently, we performed fluorescence quantification analysis on the $ChAT^+$ portions in outer hair cells (Figure 2 K-M). Likewise, we found that the total fluorescence intensity, total volume, and average fluorescence intensity remained increased in the PD group, with a much more pronounced increase in the mid-basal turn. At the level of individual outer hair cells (Supplementary Figure S3 D-F, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*), these analyses were also similar to overall outer hair cell $ChAT^+$ segment alterations. Notably, although the $ChAT^+$ portion in the hair cell region of the PD model seemed much more activated, its distribution appeared more disorganized, particularly on the apical and basal turns (Figure 2 A-C).

3.3. TH^+ fibers showed heterogeneous alterations in the PD mouse cochlea

TH, commonly used to label the morphology and short-term dopaminergic neuronal activity of dopamine

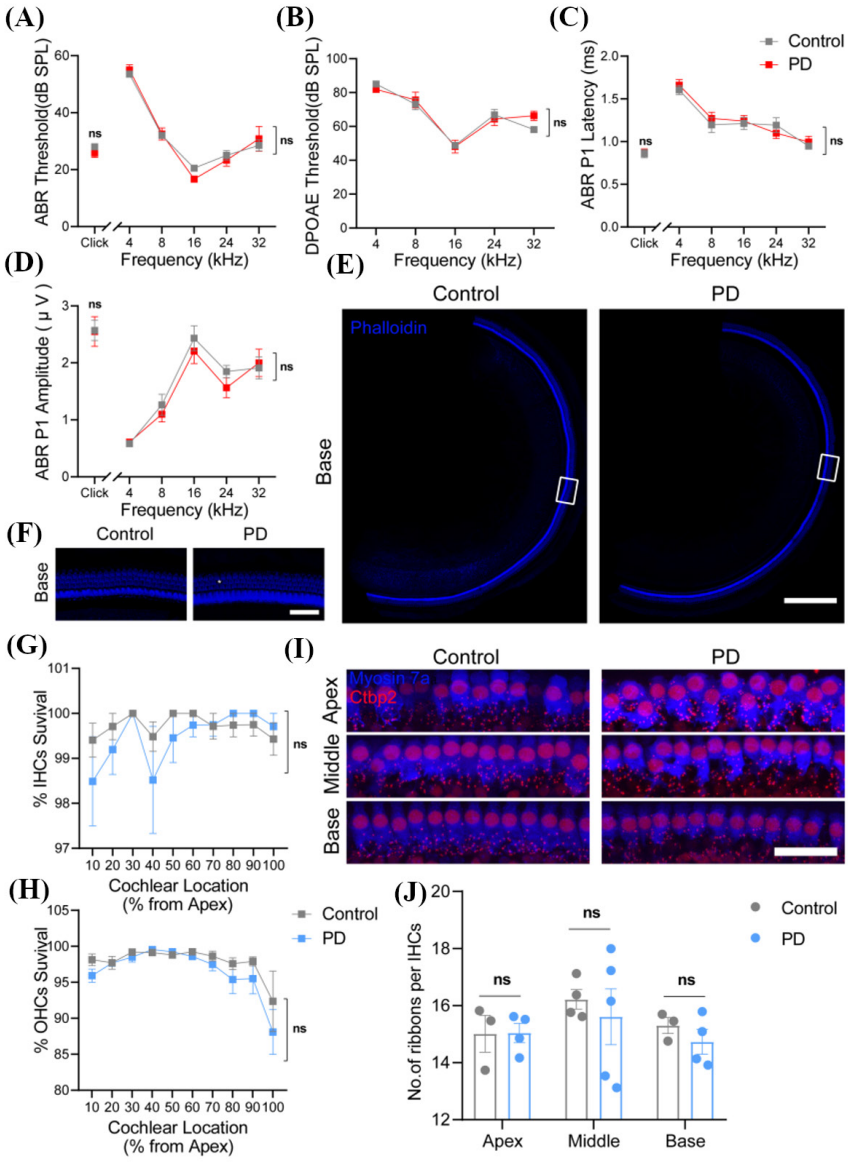

Figure 1. No significant differences were observed in hearing and corresponding morphology between PD and control mice. (A-D) Audiometric testing of control and PD mice: **(A)** showed ABR (control, *n* = 10; pd, *n* = 6); **(B)** showed DPOAE (control, *n* = 8; pd, *n* = 8); **(C)** showed ABR wave 1 latency (control, *n* = 10; pd, *n* = 6); **(D)** showed ABR wave 1 amplitude (control, *n* = 10; pd, *n* = 6). (E-H) Cochlear hair cell analysis in control and PD mice: **(E)** showed survival of basal turn hair cells, with blue labeling for phalloidin, bar = 250 μm; **(F)** was a magnification of the region in E, bar = 40 μm; **(G)** presented quantitative analysis of inner hair cells in both groups (control, *n* = 5; pd, *n* = 5); **(H)** presented quantitative analysis of inner and outer hair cells in both groups (control, *n* = 5; pd, *n* = 5). (I-J) Detection of ribbon synapses in inner hair cells of control and PD mice: **(I)** showed representative images of apical, middle, and basal turns in both groupsl; blue indicated Myosin 7a, red indicated Ctbp2, bar = 30μm; **(J)** showed quantitative analysis of apical, middle, and basal turns in both groups. Results are presented as mean ± SEM; ns indicates no significance. Two-way ANOVA followed by Sidak's multiple comparisons test.

fibers, can also mark the morphology and activity of noradrenergic fibers (*27*). TH^+ fibers in the cochlea are usually classified into dopaminergic fibers situated in the inner hair cell region and adrenergic sympathetic fibers located in the OSL (*28*). Our results showed that dopamine fiber distribution significantly decreased in the apical-middle region of the PD group, while it significantly increased in the basal turn (Figure 3B), which was verified by quantitative analysis in Figure 3 C-E. However, the average fluorescence intensity of the TH^+ region was significantly reduced, particularly in the basal turn. And in the OSL, for the first time, we observed significant reductions both in total fluorescence intensity, total volume, and average fluorescence intensity in the TH^+ region, particularly in the mid-basal turn (Figure 3A and F-H). Next, we focused on the colocalization between cholinergic and dopaminergic neurons in the inner hair cell region. The results (Figure 3 I-K) showed that PD modeling significantly altered the colocalization between them, and a marked increase was evident in the basal turn.

3.4. PD model mice exhibited heightened sensitivity to low-to-moderate noise intensity

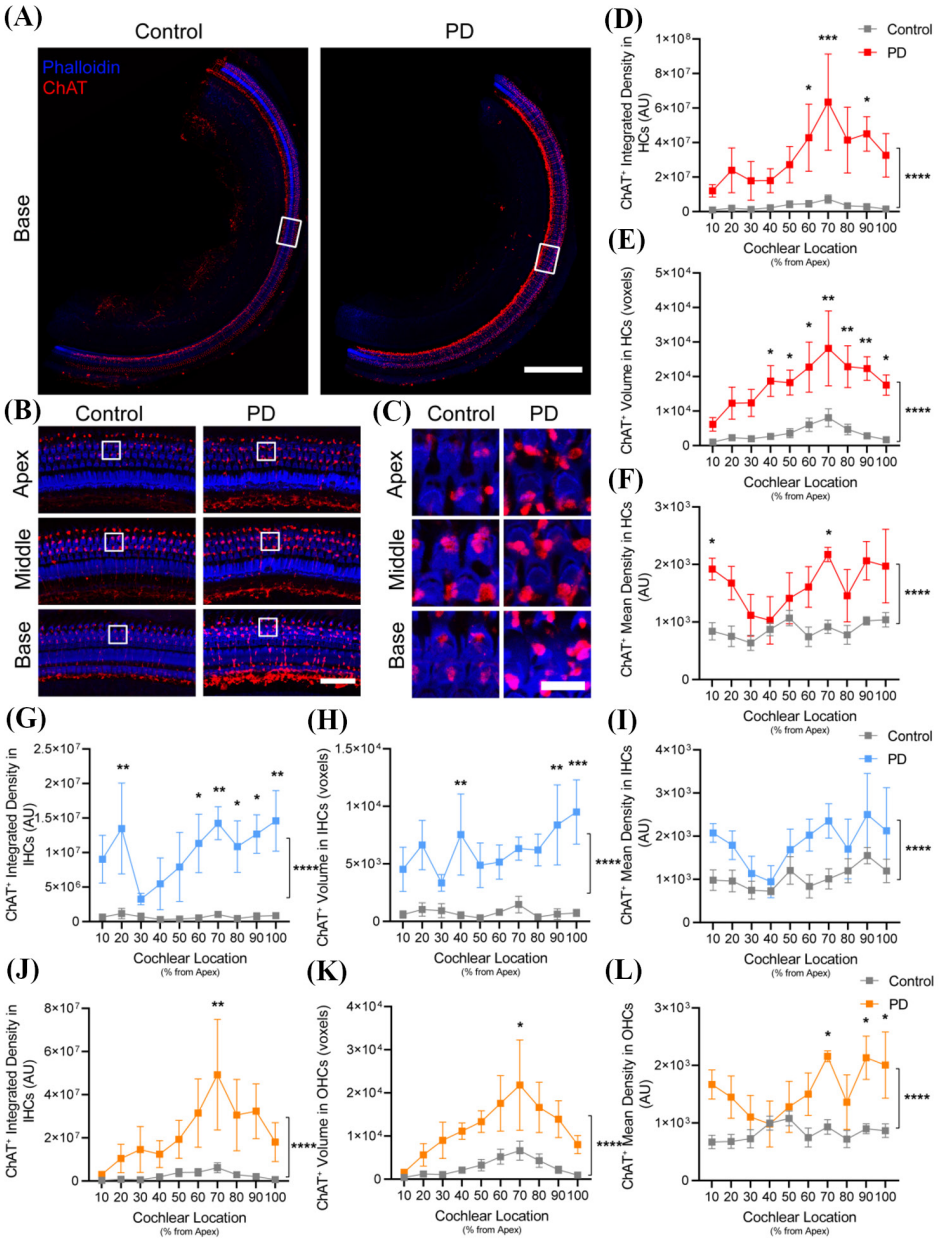

Figure 2. ChAT$^+$ fibers in PD mice cochlea exhibited post-injury compensatory changes. (A) showed the ChAT$^+$ portion of the basal turn, with blue labeling for Phalloidin and red labeling for ChAT, bar = 250 μm. **(B)** displayed the change of ChAT$^+$ portion in apical, middle, and basal turn, bar = 40 μm. **(C)** showed a magnified view of B, bar = 10 μm. **(D)(E)(F)** presented quantitative analysis of ChAT$^+$ portion in hair cells, corresponding to integrated density, volume, and mean density, respectively. **(G)(H)(I)** quantitative analysis of ChAT$^+$ portion in inner hair cells, corresponding to integrated density, volume, and mean density, respectively. **(J)(K)(L)** quantitative analysis of ChAT$^+$ portion in outer hair cells, corresponding to integrated density, volume, and mean density, respectively (control, $n = 5$; PD, $n = 4$). Results are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0001$. Two-way ANOVA followed by Sidak's multiple comparisons test.

Based on our research (*29*), we exposed both groups to 108 dB SPL white noise for 2 hours. ABR and DPOAE tests were conducted at day 1, 3, and 7 post-noise exposure to investigate hearing changes (Figure 4A). Our results revealed that at day 1 post-noise exposure, the PD group exhibited significant ABR threshold shifts compared to the control group in all frequencies, and much pronounced on clicks and 16 kHz (Figure 4B). By day 3, this gap gradually narrowed (Figure 4C), and on day 7 post-noise exposure, shift differences still existed between groups, although a significant gap was observed only in the high-frequency range (Figure 4D). But in DPOAE results, no significant differences were found, although the overall threshold shift gap between groups mirrored that of ABR (Figure 4 E-G). Interestingly, at 3 days post-noise exposure, the threshold shift at 32 kHz was much lower in the control group. And then, we examined changes in ABR wave 1 latency and amplitude (Figure 4 H-M). The PD group exhibited increased ABR wave 1 latency and decreased ABR wave 1 amplitude. Interestingly, the latency of ABR wave 1 showed no significant change at 32 kHz.

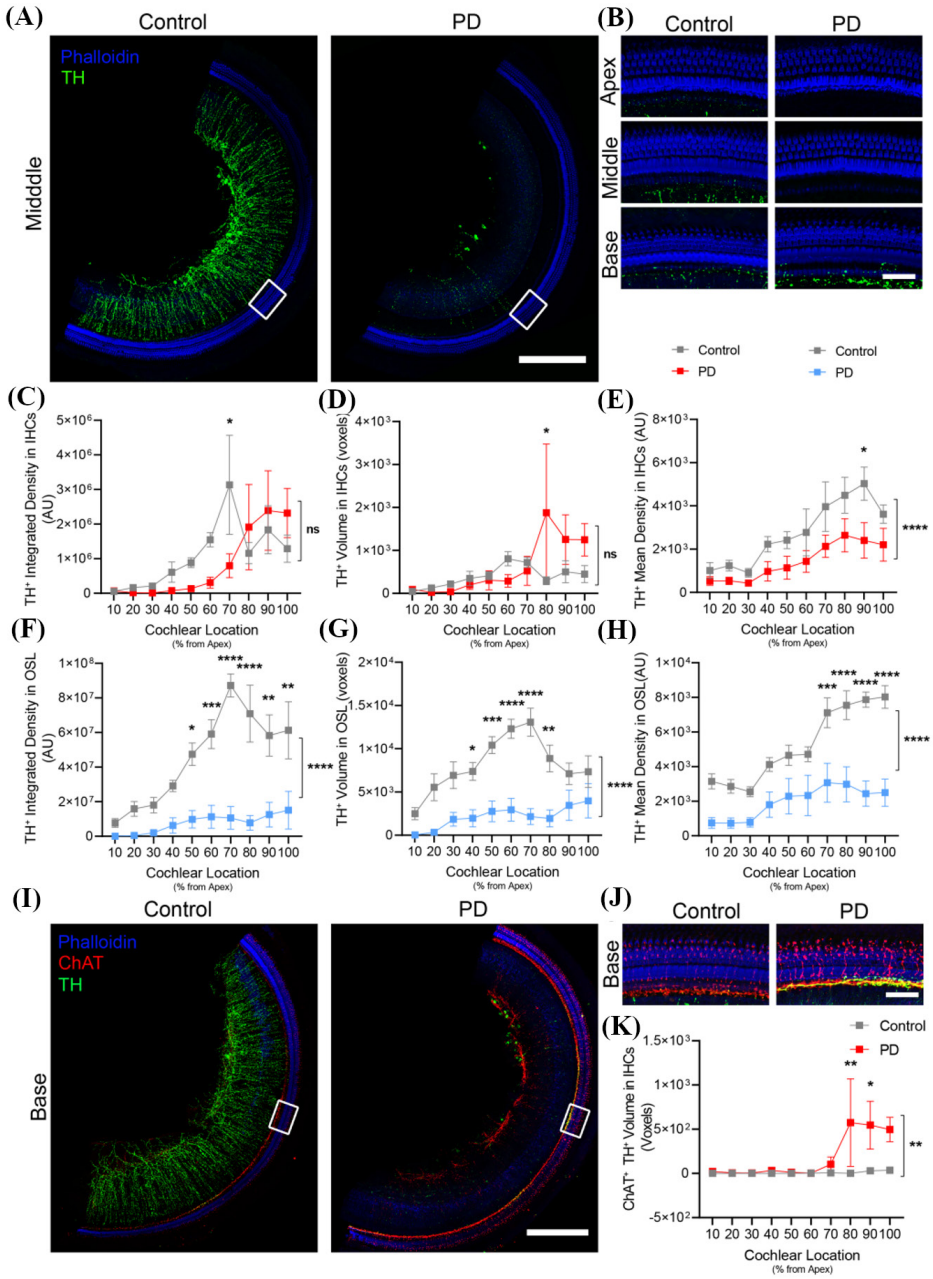

Figure 3. TH$^+$ fiber alterations in PD mouse models. (A) showed that the middle turn TH$^+$ region primarily displays sympathetic fibers in the medial spiral plate. Blue staining indicates Phalloidin, green staining indicates TH. Bar = 250 μm. **(B)** displayed the change of dopamine fibers in apical, middle, and basal turns, bar = 40 μm. **(C)(D)(E)** presented quantitative analyses of the inner hair cell TH$^+$ region, corresponding to integrated density, volume, and mean density, respectively. **(F)(G)(H)** presented quantitative analysis of the OSL TH+ region, corresponding to integrated density, volume, and mean density, respectively (control, $n = 6$; PD, $n = 5$). **(I)** showed the colocalization of ChAT$^+$ and TH$^+$ in the basal turn. Blue labeling indicated Phalloidin, red labeling indicated ChAT, and green labeling indicated TH. Bar = 250 μm. **(J)** showed a magnified view of a close-up of panel I. bar = 40 μm. **(K)** spatial co-localization analysis of ChAT$^+$ and TH$^+$ inner hair cell regions across the entire cochlea (control, $n = 5$; PD, $n = 4$). Results are presented as mean ± SEM. ns, no significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0001$. Two-way ANOVA followed by Sidak's multiple comparisons test.

3.5. PD rats demonstrated alterations in auditory function and corresponding morphology

Based on prior studies, we introduced the Sprague-Dawley rats' PD model (*23-26*). We established a PD rat model according to the experimental protocol in Figure S3A and B, and verified it by behavioral and morphological assessments. In detail, SD rats were screened for hearing abnormalities and excluded as described previously (*23-26*). Rats underwent unilateral 6-OHDA lesions of the substantia nigra pars compacta (SNc). Control rats received an equivalent injection of physiological saline containing 0.02% ascorbic acid (*23-26*). Subsequent procedures were performed according to the experimental protocol after 3 weeks. Both groups of rats underwent behavioral testing, open field test, and

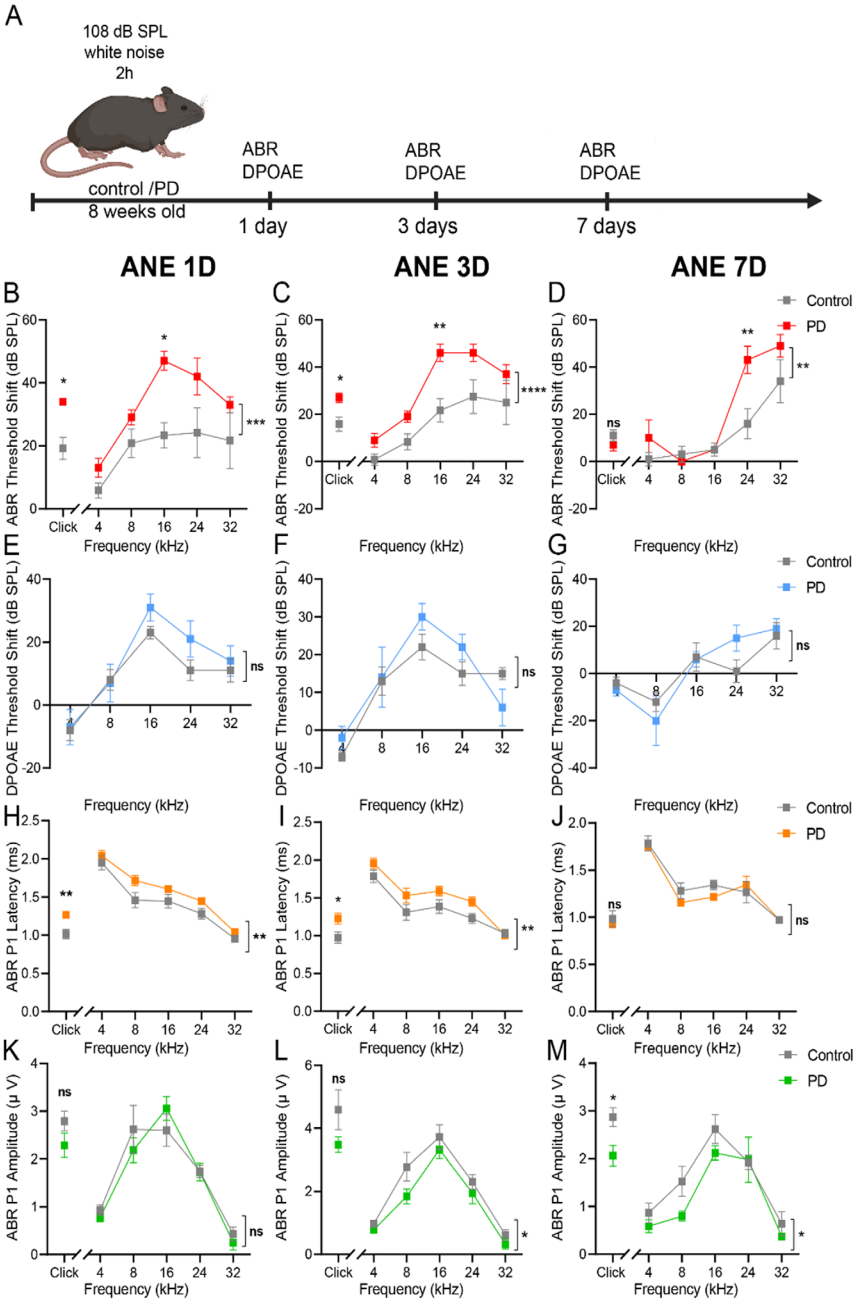

Figure 4. PD model mice exhibited heightened sensitivity to low-to-moderate noise intensity. (A) Schematic of auditory sensitivity testing. Control and PD mice underwent ABR and DPOAE testing at days 1, 3, and 7 post-exposure to 108 dB broadband white noise. **(B)(C)(D)** ABR recordings. **(H)(I)(J)** ABR L1 latency measurements. **(K)(L)(M)** ABR L amplitude measurements, (ANE 1D, control, $n = 6$; PD, $n = 5$) (ANE 3D, control, $n = 6$; PD, $n = 5$) (ANE 7D, control, $n = 5$; PD, $n = 5$). **(E)(F)(G)** DPOAE measurements (ANE 1D, control, $n = 5$; PD, $n = 5$) (ANE 3D, control, $n = 5$; PD, $n = 5$) (ANE 7D, control, $n = 5$; PD, $n = 5$). Results are presented as mean ± SEM. $*p < 0.05$, $**p < 0.01$. Two-way ANOVA followed by Sidak's multiple comparisons test.

audiological assessment, ABR and DPOAE, followed by tissue collection for pathological examination of the brain and cochlea immediately. Based on the successful establishment of the PD model, confirmed by behavioral and pathological analyses, findings regarding audiology and cochlear morphology were obtained (Supplementary Figure S2 A, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*). As shown in the results, 6-OHDA-injected rats revealed significantly impaired locomotor activity (Supplementary Figure S2 C-D, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*). Correspondingly, immunofluorescence and immunohistochemical analysis both revealed a significant reduction in dopamine neurons in the injected side of the substantia nigra in PD rats (Supplementary Figure S2 E-F, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*). Thus, we confirmed the successful establishment of the PD rat model. Considering potential bilateral effects resulting from unilateral destruction of dopamine neurons in the substantia nigra (*30,31*), we analyzed auditory and morphological parameters on

the ipsilateral and contralateral sides of PD model rats, respectively. As shown in Figure 5A, ABR thresholds in both the surgical and contralateral sides of the PD group were significantly higher than those in the control group, and similar trends were shown in DPOAE thresholds (Figure 5B). Next, we examined changes in ABR I latency and amplitude (Figure 5 C-D). Compared to the control group, both the ipsilateral and contralateral sides in the PD group exhibited prolonged latencies and reduced amplitudes significantly, in agreement with the ABR threshold shifts. Subsequently, morphological analysis among the three groups was conducted, and phalloidin staining results (Figure 5 E-H) showed no significant differences in inner hair cell survival among the three groups. However, outer hair cell survival in the control group was significantly better than in both the ipsilateral and contralateral sides of the PD group, particularly at the 10% from apex and 100% from apex positions, while the contralateral side of the PD group showed significantly reduced outer hair cell survival compared to the control group. Subsequently, we labeled the ribbon synapse, as shown in Figure 5

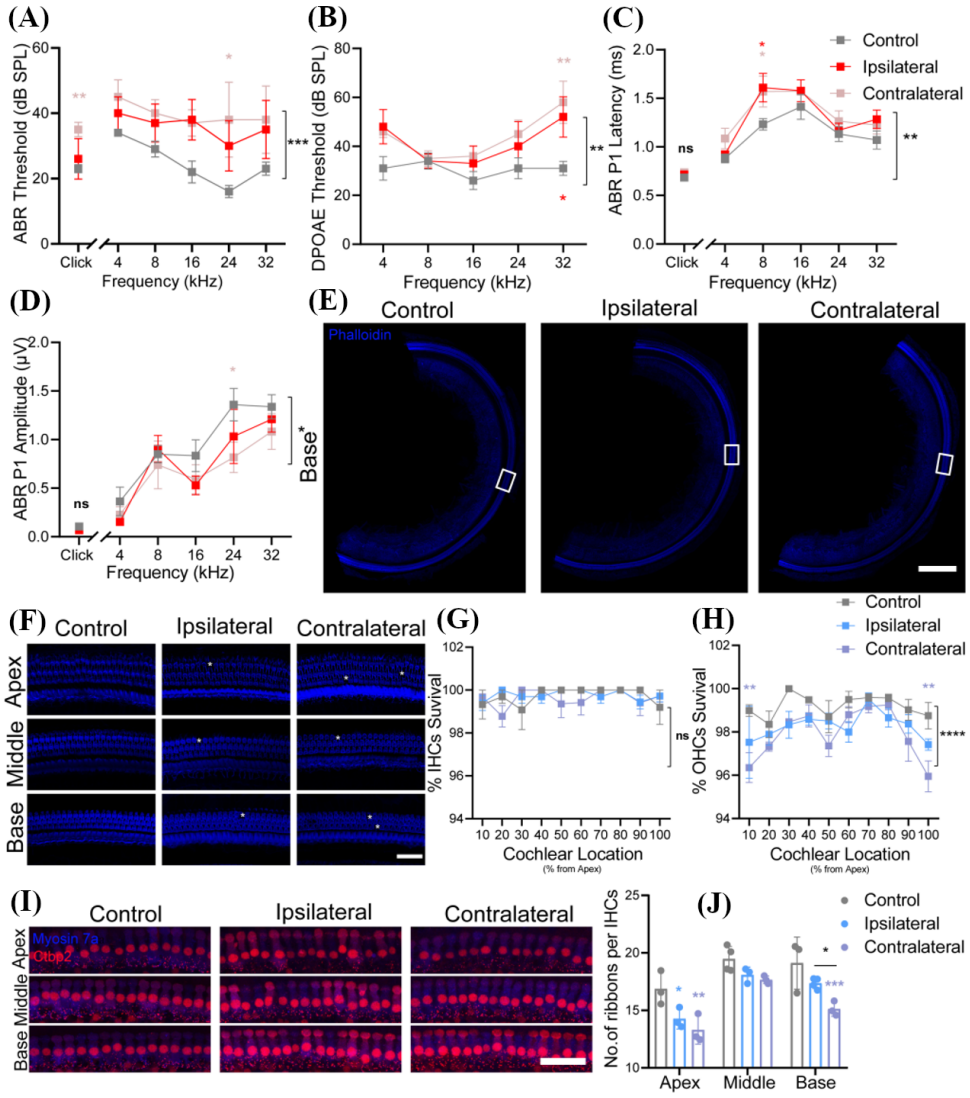

Figure 5. PD rats demonstrated alterations in auditory function and corresponding morphology. (A-D) Audiological testing of control rats and PD rats on the ipsilateral and contralateral sides: **(A)** showed ABR (control group, n = 5; ipsilateral, n = 5; contralateral, n = 5). **(B)** showed DPOAE (control group, n = 5; ipsilateral, n = 5; contralateral, n = 5). **(C)** showed ABR L1 latency (control group, n = 5; ipsilateral, n = 5; contralateral, n = 5). **(D)** showed ABR L1 amplitude (control group, n = 5; ipsilateral, n = 5; contralateral, n = 5). (E-H) Cochlear hair cell assessment in PD and control mice: **(E)** showed survival of basilar hair cells, with phalloidin labeled in blue, bar = 300 μm. **(F)** was a magnified section of E, bar = 40 μm. **(G)** presented quantitative analysis of inner hair cells in both groups (control, n = 4; ipsilateral, n = 4; contralateral, n = 4). **(H)** presented quantitative analysis of outer hair cells in both groups (control, n = 4; ipsilateral, n = 4; contralateral, n = 4). (I-J) Ribbon synapses detection in inner hair cells of PD and control mice: **(I)** showed representative images of apical, middle, and basal turns in both groups, with blue labeling for Myosin 7a and red for Ctbp2, bar = 10 μm. **(J)** presented quantitative analysis of apical, middle, and basal turns in both groups. Pink and purple p-values represented comparisons between the control group and the contralateral group, red P-values indicated comparisons between the control group and the ipsilateral group. Results are presented as mean ± SEM. ns, no significance. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.0001. Two-way ANOVA followed by Sidak's multiple comparisons test.

I-J, among the three groups, synapses at the apical, middle, and basal turns exhibited a stepwise decrease, and this reduction was more pronounced at the apical and basal turns. Notably, the number of synapses on the contralateral side was significantly lower than the control group at both apical and basal turns, coordinated with the absence of outer hair cells. Interestingly, no significant differences were observed in the arrangement of synapses or the morphology of inner hair cells among the three groups.

3.6. $ChAT^+$ fibers in PD rats displayed asymmetric alterations

We labeled the $ChAT^+$ portions of hair cells in the control group, the PD group's surgical side, and the contralateral side. Results showed that the total intensity of $ChAT^+$ portions in hair cells on both the operated and contralateral sides was higher than in the control group, and their distributions were not consistent bilaterally, although lacking statistical significance. And no significant differences were observed in the volumes of the three groups. Regarding mean fluorescence intensity, both the operated and contralateral sides were significantly higher than the control group, especially in the apical and basal regions, the contralateral side exhibited higher intensity than the operated side, although with no statistical significance (Figure 6 D-F). Next, we investigated ChAT expression in inner hair cells. The trends for total and average fluorescence intensity in the inner hair cell region were similar to the entire hair cells. However, the volume of both the ipsilateral and contralateral sides was higher than the control group, and the distribution of the two sides was inconsistent but not significant (Figure 6 G-I). In the level of individual inner hair cells, the changes in $ChAT^+$ total fluorescence intensity, total volume, and mean fluorescence intensity were similar to those observed in the entire $ChAT^+$ inner hair cell part (Supplementary Figure S2 A-C, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*). And in the outer hair cell region, total fluorescence intensity and average fluorescence intensity on both the surgical and contralateral sides of the PD group were mildly higher, while the overall volume was lower than the control group, but insignificant compared with the $ChAT^+$ region in inner hair cells (Figure 6 J-L). And individual outer hair cells showed similar results (Supplementary Figure S4 D-F, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=279*). Overall, the expression of ChAT exhibited asymmetric changes in both sides of PD rats, with more pronounced alterations in average fluorescence intensity, especially in the inner hair cell part. Furthermore, similar to the findings in mice, its distribution within inner and outer hair cells appeared more disorganized in the PD rat cochlea (Figure 6 A-C).

3.7. TH^+ fibers revealed asymmetric alterations in the PD rat model

Then, we labeled dopaminergic neurons in the inner hair cell region and adrenergic sympathetic fibers in the OSL region using TH labeling. As shown in Figure 7 B-E, no significant differences were observed in the total fluorescence intensity or volume of TH^+ regions within the inner hair cell area among the groups. However, in the region 70% from the apex, the total fluorescence intensity in the PD group's ipsilateral and contralateral sides was higher than the control group, with significance observed only between the PD group's ipsilateral side and the control group. Regarding total volume, the PD group's contralateral side significantly exceeded both the control group and the operated side. In terms of average fluorescence intensity, both the surgical and contralateral sides of the PD group exhibited higher values compared to the control group. However, the relationship among the three groups was not uniform. In the region 40–50% from the apex, the average TH fluorescence intensity on the contralateral side of the PD group was significantly higher than the control group. Conversely, in the regions 70–90% from the apex, the PD group's operated side showed higher TH average fluorescence intensity than the control group, where the changes in the PD group's contralateral side tended toward the control group's values. Next, we examined the TH^+ fraction in the OSL region (Figure 7A and F-H). Results revealed no significant differences in total intensity or volume among the three groups in the regions 10–60% from the apex. However, in the 60–100% region, the control group, the PD contralateral side, and the PD surgical side exhibited a stepwise decrease, with volume showing a significant reduction. Nevertheless, no significant differences were observed in mean fluorescence intensity among the three groups. In contrast, changes in the TH^+ region exhibited more complex characteristics in the 6-OHDA rat model group. Subsequently, we examined co-localization between $ChAT^+$ and TH^+ parts in the inner hair cell region of rats. Results revealed that PD modeling reduced co-localization probability, particularly in the region 70% from the apex, where the PD group's surgical side showed significantly lower levels than the control group. At this point, the PD group also showed significantly lower levels on the operated side compared to the contralateral side. Although the contralateral side in the PD group was lower than the control group, the difference was not statistically significant. However, notably, compared to mice, the overall colocalization voxels in the rat model were not high (Figure 7 I-K).

4. Discussion

Growing evidence suggests that hearing loss, as a non-motor sensory impairment in PD, may serve as an indicator of progression and treatment response (*32*).

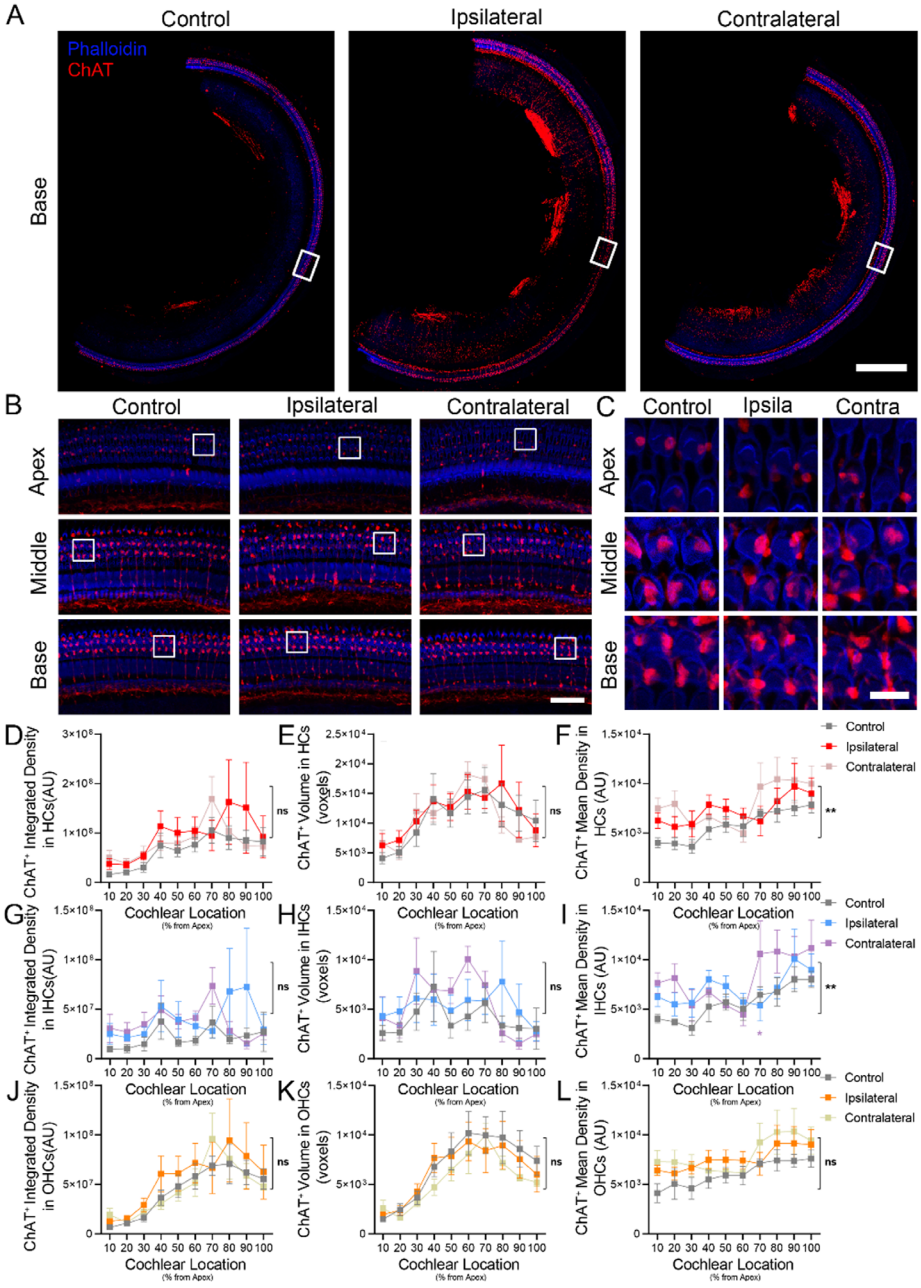

Figure 6. ChAT$^+$ fibers in PD rats displayed asymmetric alterations. (A) showed the ChAT$^+$ portion of the basal turn, with blue labeling for Phalloidin and red labeling for ChAT, bar = 300 μm. **(B)** displayed the change of ChAT$^+$ portion in apical, middle, and basal turn, bar = 40 μm. **(C)** is a magnification of panel B, bar = 10 μm. **(D)(E)(F)** presented quantitative analysis of ChAT$^+$ portion in hair cells, corresponding to integrated density, volume, and mean density, respectively. **(G)(H)(I)** quantitative analysis of ChAT$^+$ portion in inner hair cells, corresponding to integrated density, volume, and mean density, respectively. **(J)(K)(L)** quantitative analysis of ChAT$^+$ portion in outer hair cells, corresponding to integrated density, volume, and mean density, respectively (control, $n = 4$; ipsilateral, $n = 4$; contralateral, $n = 4$). Purple p-values represented comparisons between control and contralateral groups. Results are presented as mean ± SEM. ns, no significance. *$p < 0.05$, **$p < 0.01$. Two-way ANOVA followed by Sidak's multiple comparisons test.

However, studies on peripheral, particularly cochlear, pathological manifestations and mechanisms in PD models remain scarce. Therefore, this work primarily focuses on hearing changes and cochlear pathology in PD models, aiming to provide new insights into the mechanisms and treatment of hearing alterations in PD.

MPTP, a neurotoxin commonly used to induce PD models, selectively targets dopaminergic neurons (*33*). Previous studies demonstrated that MPTP significantly alters auditory measures such as ABR and category of auditory performance (CAP), causes marked damage to central auditory structures, including the inferior colliculus and lateral colliculus, and severely disrupts the echolocation system in bats (*34,35*). However, in our current study, MPTP-treated mice exhibited no clear alterations in ABR or DPOAE, while no significant loss of inner or outer hair cells was observed, hinting that MPTP exposure alone may be insufficient to induce pronounced peripheral auditory dysfunction in our PD mice. Ribbon synapses, the critical structures that

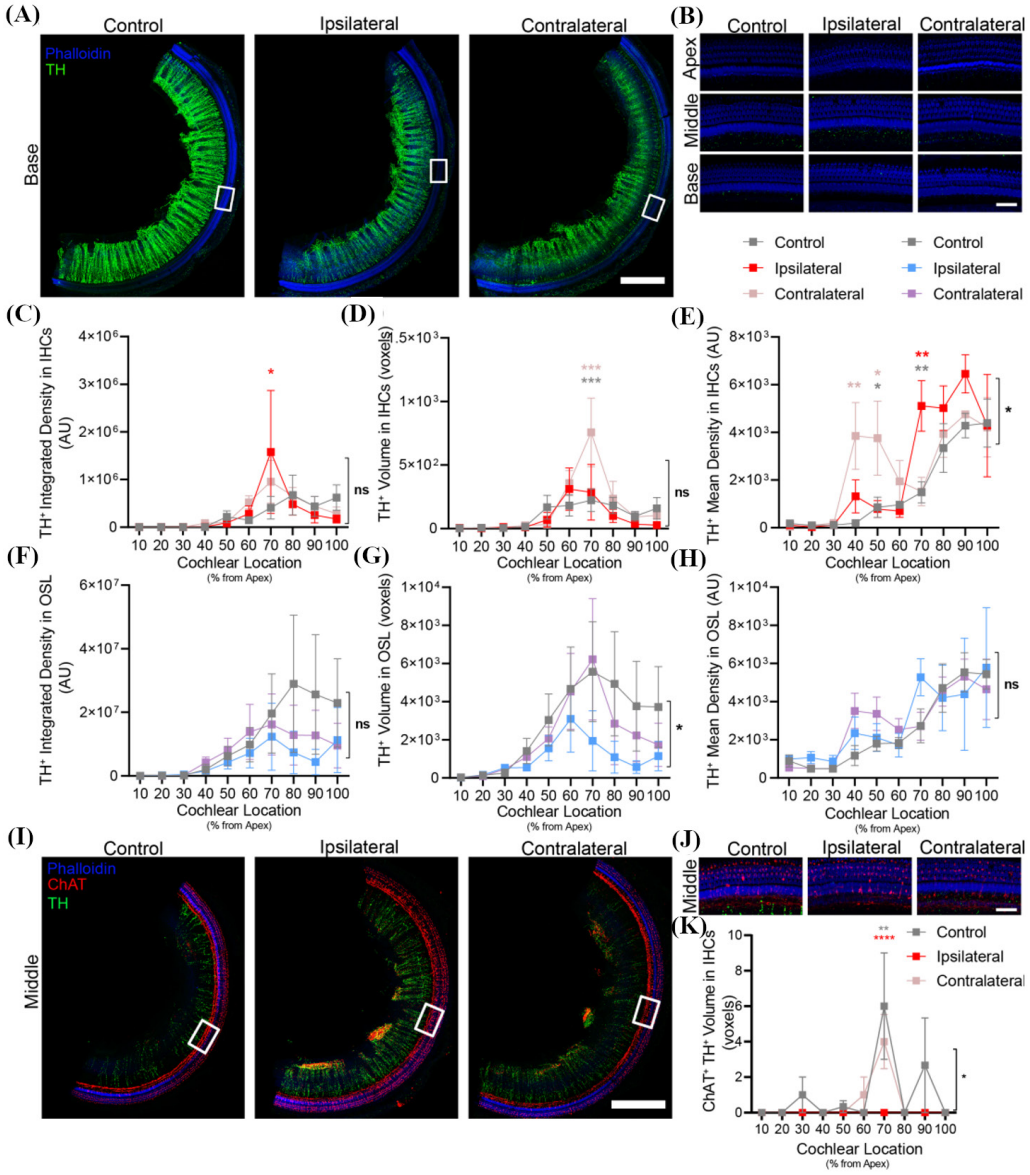

Figure 7. TH$^+$ fibers revealed asymmetric alterations in the PD rat model. (A) The middle turn TH$^+$ region primarily displays sympathetic fibers in the medial spiral plate. Blue staining indicates Phalloidin, green staining indicates TH. bar = 300 μm. **(B)** displayed the change of dopamine fibers in apical, middle, and basal turns, bar = 40 μm. **(C)(D)(E)** presented quantitative analyses of the inner hair cell TH$^+$ region, corresponding to integrated density, volume, and mean density, respectively. **(F)(G)(H)** presented quantitative analysis of the OSL TH$^+$ region, corresponding to integrated density, volume, and mean density, respectively (control, n = 3; lpsilateral, n = 3; contralateral, n = 3). **(I)** showed the colocalization of ChAT$^+$ and TH$^+$ in the middle turn. Blue labeling indicated Phalloidin, red labeling indicated ChAT, and green labeling indicated TH. bar = 300 μm. **(J)** showed a magnified view of a close-up of panel. bar = 40 μm. **(K)** Spatial co-localization analysis of ChAT$^+$ and TH$^+$ inner hair cell regions across the entire cochlea (control, n = 3; ipsilateral, n = 3; contralateral, n = 3). Red p-values indicated comparisons between the control and ipsilateral groups. Gray p-values indicated comparisons between the ipsilateral and contralateral groups. Results are presented as mean ± SEM. ns, no significance. *$p < 0.05$, **$p < 0.001$, ***$p < 0.005$. Two-way ANOVA followed by Sidak's multiple comparisons test.

transmit signals from inner hair cells to afferent neurons, are known to exhibit early pathological changes that precede alterations in ABR, DPOAE thresholds, and hair cell survival, particularly in hidden hearing loss (*36*). Notably, our study found no significant loss of synapses in all turns. Consistently, the latency and amplitude of the ABR 1 wave showed no obvious changes compared to controls. Interestingly, despite no clear numerical change, the synapses at the basal turn exhibited greater regularity relative to those at the apical and middle turns. Correspondingly, the ABR wave 1 amplitude at 32 kHz remained largely unchanged compared to 8 kHz and 16 kHz. These findings suggest that MPTP did not specifically target hair cells or synapses to cause significant damage. Interestingly, previous studies have found a higher proportion of functional hearing loss among PD patients, where no significant differences in pure-tone audiometry (PTA) abnormalities were observed (*37*).

The LOC and MOC systems, as efferent components of the auditory pathway, play a critical role in auditory sensitivity and discrimination. Alterations in these

systems often precede hair cells and ribbon synapses (*38*). In our PD models, ChAT-labeled cholinergic neurons exhibited a marked increase in both fiber volume and ChAT expression levels, indicating enhanced cholinergic activity, which may reflect secondary or compensatory mechanisms. Moreover, we noted more disorganized ChAT distribution at the apical and basal turns, further indicating that compensatory activity of cholinergic fibers is possibly dysregulated. Interestingly, previous studies involving MPTP injection into the guinea pig round window showed no significant changes in MOC volume. However, this study primarily focuses on MPTP's toxicological effects rather than its potential damage to the systemic motor and non-motor systems as a PD model inducer (*35*).

As a key component of the cochlear LOC, dopaminergic neurons primarily coordinate with cholinergic neurons in the LOC to encode sound localization in inner hair cells. Previous studies indicated that dopaminergic neurons in the LOC may form synaptic structures with auditory afferents, inhibiting the excitability of auditory afferent electrical activity (*11*). To date, no studies have focused on their potential alterations in PD models. We report, for the first time, that dopamine fibers in the apical and middle turn are significantly depleted in the MPTP model. Therefore, we hypothesize that loss of dopaminergic fibers disrupts inhibitory control within the LOC circuitry, leading to compensatory activation of cholinergic neurons; however, as we have seen, this activation is not perfect, which failed to shield inner hair cells and synapses from the risk of glutamate toxicity caused by an abnormal increase in afferent signals. Consequently, the body rapidly but imperfectly activates the MOC efferent system, the myelinated fiber, to inhibit the excitability of outer hair cells and reduce the intensity of sound signal input, ultimately protecting inner hair cells and synaptic structures (*10*). Interestingly, beaded enlargements of dopaminergic fibers were observed at the basilar turn of the cochlea. However, it remains unclear whether these swollen dopaminergic neurons retain partial functionality. Notably, dopaminergic and cholinergic neurons show marked co-localization increases in the basilar turn. Previous studies also suggest their increased overlap after noise exposure (*13,27*), though their precise significance remains unclear. Interestingly, we observed a marked loss of adrenergic sympathetic innervation in the OSL for the first time. Similar denervation of sympathetic fibers has been observed in cortical and cardiac tissues of MPTP-induced PD monkey models (*39,40*). This absence may result from the secondary consequences of dopaminergic degeneration in the substantia nigra. Numerous studies have demonstrated concomitant sympathetic fiber pathology in PD patients (*41,42*). In the cochlea, sympathetic fibers in the OSL primarily regulate vascular tone supplying hair cells and afferent/efferent nerves (*43*). Following sympathetic denervation, the loss of vascular tone may leave the hair cells and afferent/efferent nerves in a relatively ischemic state; however, its specific role in hearing remains unclear.

Based on these findings and prior research, we hypothesized that our MPTP-induced mouse model primarily caused damage in auditory sensitivity (*44*). Therefore, we exposed PD mouse models to low-to-moderate intensity noise. Noise exposure for 1, 3, and 7 days resulted in significant fluctuations in ABR thresholds, P1 latency, and amplitude, while DPOAE exhibited no significant change, suggesting that outer hair cells may not have sustained substantial damage. These findings validate our hypothesis that in our mouse model, hearing impairment primarily manifested as changes in auditory sensitivity resulting from the loss of compensation in the LOC and MOC. We also observed that, compared to other frequencies, the amplitude of fluctuations at 32 kHz was not significantly affected by low-to-moderate noise exposure, particularly evident in ABR P1 latency. This suggested that the beaded dopamine neurons located at the basilar turn, identified in our results, may retain partial compensatory capacity. Interestingly, previous studies on cholinergic neurons in auditory sensitivity have shown that enhancing cholinergic receptor activity at the base of outer hair cells *via* α9 nicotinic receptor knock-in reduced ABR fluctuations following low-intensity noise exposure (*45*). However, in our study, cholinergic nerve activation did not effectively rescue noise-induced auditory changes. This suggests the critical role of dopaminergic nerves in this process, while also indicating that cholinergic nerves cannot adequately compensate, as shown in our work, with the activated but disorganized cholinergic nerves.

Notably, the change in auditory sensitivity did not constitute conventional hearing loss, evidenced by significant alterations in ABR thresholds, DPOAE thresholds, or marked reductions in ribbon synapse numbers. Instead, it primarily manifested as alterations in cochlear efferent nerve fibers and amplified following mild to moderate noise exposure, as we have observed. However, this could not be described as the no differences in hearing and corresponding morphologic changes in mice. We interpret this phenomenon more as an early stage of hearing impairment based on the MPTP-induced PD mouse model. Research has demonstrated that functional hearing impairment is prevalent among PD patients, which precedes conventionally defined significant auditory changes (*37*). Some studies interpret hearing loss as a precursor to olfactory impairment (*3,46*). However, in our model, dopaminergic neurons in the substantia nigra are already significantly depleted. Therefore, interpreting the non-significant hearing impairment observed in our model as an early stage of PD-related hearing loss appears more appropriate, based on the MPTP-induced PD mice model.

Increasing clinical data have reported the lateralization of cochlear dysfunction in PD patients

(*5*). To elucidate the potential peripheral mechanisms underlying this phenomenon, we introduced 6-OHDA rat models with unilateral substantia nigra lesions. Notably, compared to bilateral 6-OHDA-induced rats, 6-OHDA-induced rats appear to be much more common in studies investigating the lateralization of PD (*46,47*). Furthermore, unilateral 6-OHDA injection yields higher postoperative survival rates (*48,49*), which is why we adopted this model. Furthermore, considering the potential risk of methamphetamine-induced hearing loss and the necessity of verifying successful model establishment through its-induced rotational movements (*23,24,50-52*), we adopted the more appropriate open-field test. Combined with the pathological feature of significant loss of dopaminergic neurons in the unilateral substantia nigra, this confirmed the successful establishment of the model.

Notably, compared to mouse PD models, where changes in ABR, DPOAE, hair cells, and synapses were insignificant, the PD rat model exhibited more pronounced alterations. Additionally, we also observed lateralization of peripheral auditory changes in the unilateral 6-OHDA-induced PD rat that hearing in the contralateral ear appeared worse than in the ipsilateral ear, followed by greater loss in hair cells and synapses of apical and basilar turns in the contralateral cochlea.

Previous studies have suggested that MOC and LOC may contribute to unilateral hearing loss in PD (*32*). Therefore, we labeled the LOC and MOC systems in rats. Interestingly, compared to PD mouse models, cholinergic neurons in the MOC showed no significant alterations, whereas those in the LOC exhibited bilateral asymmetric changes. A similar pattern of asymmetry was also observed in dopaminergic neurons. Unexpectedly, dopaminergic neurons displayed asymmetric activation rather than the pronounced structural and functional inhibition observed in mice, which may correlate with the 6-OHDA modeling approach (*26*). Increasing studies suggested that nigrostriatal dopaminergic neurons primarily innervate the auditory system through corresponding dopamine receptors or other types of nerve fibers (*10,15*), and the auditory system possesses an independent dopaminergic system separate from the nigrostriatal pathway (*53*). Following the death of substantia nigra dopaminergic neurons, the overall dopamine levels decrease, potentially leading to compensatory activation of dopaminergic neurons in the auditory system. However, due to the reciprocal innervation between the nigrostriatal and auditory systems, this compensation may be asymmetric (*32*). This compensation not limited to dopaminergic neuron, studies indicate complex connections also exist between cholinergic neurons and the auditory system (*54*). Furthermore, recent research suggests direct links may exist between basal ganglia regions, including the substantia nigra, and the auditory system (*15*). Consequently, unilateral nigral dopamine depletion may induce alterations in the central auditory system, which, together with ipsilateral compensatory changes in the LOC and MOC systems, could influence peripheral hearing thresholds and susceptibility. However, whether these LOC and MOC changes are secondary to nigral dopamine lesions or occur concurrently remains inconclusive. Additionally, we observed colocalization of cholinergic and dopaminergic elements within the LOC system showed bilateral asymmetric changes. Notably, although the sympathetic nerve located in the OSL did not show the marked depletion observed in mouse models, its reduction was still more pronounced on the surgical side, which aligns with our hypothesis that the central auditory system contributes to peripheral hearing pathology in PD models possibly.

Compared to the more extensive pathological damage observed in MPTP mouse models — including bilateral substantia nigra dopamine depletion, dopaminergic fibers loss in the cochlea, sympathetic fibers loss, and widespread cholinergic neuron activation, the 6-OHDA rat model — characterized by unilateral substantia nigra dopaminergic damage and bilateral asymmetric yet mild cholinergic and dopaminergic alterations — appears to correlate with more severe audiological and corresponding pathological changes. This discrepancy may be partly due to differences in molding methods and medications. Moreover, due to cross-innervation and functional complementarity between the central and peripheral nervous systems, unilateral substantia nigra dopamine depletion seems to elicit widespread compensatory mechanisms across both domains. Such compensatory responses are particularly common in neural lesion models (*55*).

5. Conclusion

In summary, we presented, for the first time, hearing alterations, pathological features, and potential mechanisms in the cochlea of MPTP-induced mouse and 6-OHDA-induced rat PD models, with particular focus on the role of efferent fibers. Furthermore, we, for the first time, reported denervation of adrenergic sympathetic fibers and ipsilateral peripheral hearing loss in MPTP-induced mouse and 6-OHDA-induced rat PD models, respectively. However, this study did not definitively explain whether loss of adrenergic sympathetic fibers in the MPTP model is a consequence of substantia nigra dopaminergic lesions. And due to the complexity of research involving both central auditory pathways and substantia nigra dopaminergic systems, we were unable to conduct more detailed investigations into central auditory changes and their secondary effects on peripheral hearing in the 6-OHDA-induced rat model. Furthermore, the pathogenesis of PD appears to be more complex, and currently, there is no PD animal model that can explain the total clinical progression in PD perfectly. Beyond the two classic models mentioned in our work,

additional models exist to simulate PD at different stages and under varying pathological conditions, which may also exhibit varying degrees of auditory changes but need further investigation. Thus, our findings were based only on studies using two classical animal models of PD and could not be fully equated with the clinical manifestations observed in PD patients. Moreover, most current clinical hearing studies in PD patients rely on tests like PTA, which can only evaluate significant hearing loss but not central auditory damage, hidden hearing loss, and functional hearing impairment. Further research should focus on these types of hearing impairment in clinical PD patients. In addition, age and other diseases causing cognitive impairment may confound hearing loss in PD patients (*56,57*). These factors limited our interpretation of many findings observed in this work.

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