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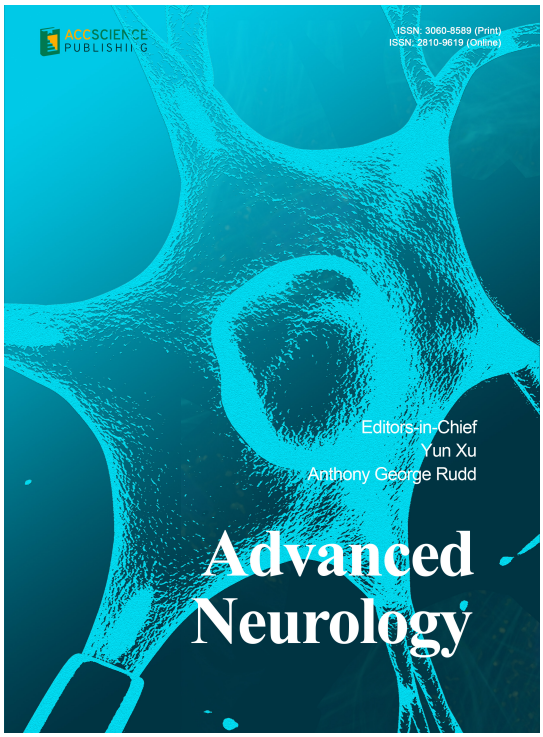
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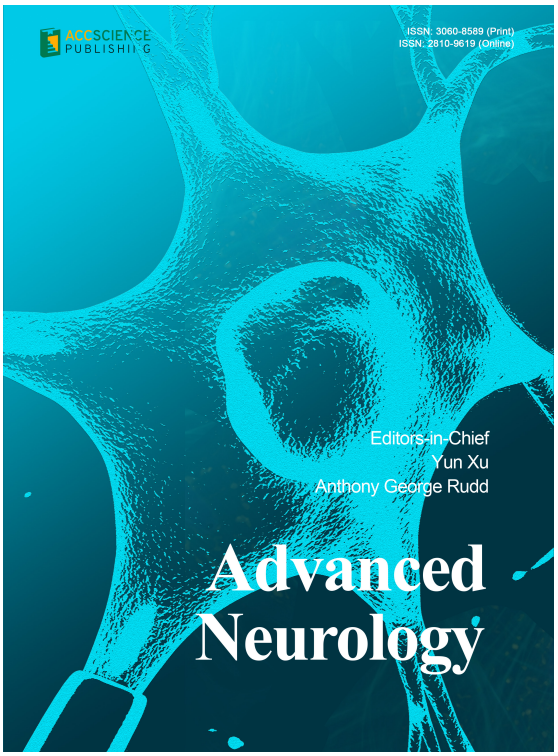
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A close-up graphic image of a neuron

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Volume 1 | Issue 3 | December 2022 |

Advanced Neurology

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CONTENTS

1 **Tracking of transplanted neural stem cells labeled with superparamagnetic iron oxide in ischemic stroke** *ORIGINAL RESEARCH ARTICLE*
Ying Bai, Zhongqiu Zhou, Fengchao Zang, Ling Shen, Yuan Zhang, Shuo Leng, Guangtian Wang, Bing Han
https://doi.org/10.36922/an.v1i3.278

2 ***Emx1-Cre*-mediated inactivation of PDK1 prevents plaque deposition in an Alzheimer's disease-like mouse model** *ORIGINAL RESEARCH ARTICLE*
Xiaolian Ye, Liyang Yao, Wenhao Chen, Wenkai Shao, Yimin Hu, Bing Zhang, Guiquan Chen
https://doi.org/10.36922/an.v1i3.153

3 **Gut-brain axis in depression: Crosstalk between neuroinflammation and gut microbiota** *REVIEW ARTICLE*
Yang Cai, Shenyang Zhang, Sibo Zhao, Honghong Yao
https://doi.org/10.36922/an.v1i3.272

4 **Anti-leucine-rich glioma inactivated-1 autoimmune encephalitis: A review of diagnosis and treatment** *REVIEW ARTICLE*
Ning Gu, Tian-Yue Meng, Zhuang Zhu, Bao-Xin Wu, Ying-Feng Mu, De-Qin Geng
https://doi.org/10.36922/an.v1i3.237

5 **Potential therapeutic targets and medications for arteriovenous malformations of the central nervous system** *REVIEW ARTICLE*
Zhengsong Li, Yueshan Feng, Shiju Zhang, Yuan Zhou, Jiaxing Yu, Hongqi Zhang, Tao Hong
https://doi.org/10.36922/an.v1i3.211

6 **Brief risk rating scale: A preliminary screening and monitoring tool emphasizing individual differences for better prognosis in Alzheimer's disease** *REVIEW ARTICLE*
Qiujie Shan, Ping-Hsuan Wei, Yun Xu, Feng Bai
https://doi.org/10.36922/an.v1i3.208

7 **Mechanism of action and therapeutic targeting of microglia in autism spectrum disorder** *REVIEW ARTICLE*
Yangming Zhang, Yuqiao Xie, Zishuo Cheng, Yanran Zhang, Wenting Wang, Baolin Guo, Shengxi Wu
https://doi.org/10.36922/an.v1i3.167

8 **Vertigo or dizziness as the first presentation of epilepsy: A case report** *CASE REPORT*
Ning Wu, Xiaojun Zhang, Yanqiang Wang
https://doi.org/10.36922/an.v1i3.140

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Advanced Neurology

ORIGINAL RESEARCH ARTICLE

Tracking of transplanted neural stem cells labeled with superparamagnetic iron oxide in ischemic stroke

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Abstract

Clinical treatments for ischemic stroke are limited. At present, neural stem cell (NSC) therapy is considered a promising method for treating ischemic stroke. However, the behavior of transplanted NSCs remains ambiguous. Herein, we report the feasibility of superparamagnetic iron oxide labeling for long-time magnetic resonance imaging to track transplanted NSCs in a transient middle cerebral artery occlusion model. According to our studies, *in vivo* monitoring of transplanted NSCs during ischemic stroke in the current work may provide another insight into the tracking strategy of transplanted NSCs, thereby advancing NSC-based therapy toward clinical application.

Keywords: Neural stem cell; Ischemic stroke; Superparamagnetic iron oxide; Magnetic resonance imaging; Tracking

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1. Introduction

Ischemic stroke causes significant mortality and morbidity, affecting millions of people worldwide[1-3]. Given the potential risk factors and the aging population, the prevalence of ischemic stroke is expected to increase[4]. Therapeutic strategies for stroke have made significant progress during the past several years. Current pharmacological treatments for ischemic stroke are primarily to reduce brain damage through the use of thrombolytic agents, antithrombotic agents, or neuroprotective agents[5-7]. However, the current therapies cannot be applied to all ischemic stroke patients and adequately improve the outcomes.

Studies have shown that the use of cell therapy following ischemic stroke increases neuroplasticity, improves the structure and physiological functions of brain tissue, and promotes the recovery of the nervous system[8]. Neural stem cells (NSCs) play key roles in brain homeostasis and exhibit potential therapeutic function in ischemic stroke[5,9-11]. Our previous study has demonstrated that si-circHIPK2 regulates NSC differentiation, and NSCs transduced with si-circHIPK2 promote neuroprotection and functional recovery after stroke[12]. However, the clinical application of stem cell therapy remains a big challenge because the biodistribution and fate of transplanted stem cells in the brain need to be determined in this therapy[13,14]. Tracking transplanted NSCs *in vivo* to eval-

uate their effect on ischemic stroke is the key to evaluating the treatment strategy for ischemic stroke.

In recent years, a number of methods have been used to track NSCs, such as bromodeoxyuridine (BrdU)[15]. However, this method has its limitations because BrdU signal becomes weak when transplanted NSCs proliferate and differentiate, making it difficult to monitor the cells over a long time. Researchers are now making efforts to develop appropriate methods for long-term tracking of stem cells after transplantation. Here, superparamagnetic iron oxide (SPIO) nanoparticles are considered to be the practical application of stem cells because nano iron oxide is the only inorganic nanomaterial that has been approved by the Food and Drug Administration (FDA) as a nanodrug[16]. SPIO can be used as a clinical magnetic resonance imaging (MRI) contrast agent[17,18]. Studies have shown that SPIO has been successfully used for tracking different cell types by MRI *in vivo* because of its characteristics, which include easy synthesis, superparamagnetic, high saturation magnetization, good biocompatibility, and low toxicity[19]. To monitor the migration and survival of transplanted NSCs *in vivo*, SPIO can be used as an MRI tracer to non-invasively monitor *in vivo*.

In the present study, SPIO nanoparticles were used to detect the migration and distribution of transplanted NSCs in (transient middle cerebral artery occlusion [tMCAO]) mice. We sought to demonstrate the authenticity of SPIO-labeled NSCs tracking in tMCAO mice and the capacity of NSCs for migration, thus providing a visual basis for evaluating their therapeutic effect on ischemic stroke.

2. Materials and methods

2.1. Synthesis of SPIO nanoparticles

SPIO was synthesized based on previous reports[16,20]. First, polyglucose sorbitol carboxymethyl ether (PSC, 200 mg) aqueous solution (10 mL) was aerated with nitrogen to remove oxygen for 5 min. Second, ferric chloride ($FeCl_3$, 60 mg, 0.37 mmol) and ferrous chloride ($FeCl_2$, 30 mg, 0.236 mmol) were dissolved in 15 mL of double distilled water (ddH_2O). The mixture was then added into the PSC solution. Third, 28% of ammonium hydroxide (1 g) was added into the solution with the mixture, and then mechanically stirred in a water bath for 30 min (80°C). Next, the iron oxide was collected by an ultrafiltration centrifuge tube (30 kD) and washed with ultrapure water 3 times. The technique was based on the preparation process of ferumoxytol, which has been approved by the FDA as an inorganic nanodrug.

2.2. Inductively coupled plasma mass spectrometry (ICP-MS)

NSCs were digested into single-cell suspension by enzyme and cultured at a density of 5×10^4/well in 500 μL of medium into 24-well microplate. Then, the cells were harvested and counted. The cell suspension was dissociated by hydrochloric acid (HCl). The concentration of iron in cell lysates was measured by ICP-MS according to Perkin Elmer's operating procedures.

2.3. Ethics approval and consent to participate

All animal experiments were performed in accordance with the standard guidelines for the care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee of the Medical School of Southeast University.

2.4. Cell cultures

NSCs from C57BL/6J or enhanced green fluorescent protein (eGFP) transgenic mice were isolated from embryonic day 14 mice hippocampus and sterilized with 75% of alcohol. Fetal mice were taken out from pregnant mice and placed in a 50 mL centrifuge tube with sterile phosphate-buffered saline (PBS). Brains were removed from the fetal mice and dissociated to obtain the hippocampus. Next, the hippocampus was placed in a medium supplemented with PBS, and the tissues were digested with trypsin (25200056, Gibco, USA). Then, the cells were plated on cell culture flasks containing basic fibroblast growth factor (bFGF, 20 ng/mL, Stem Cell, Canada), epidermal growth factor (EGF, 20 ng/mL, Stem Cell, Canada), and B-27 supplement (2%, Gibco, USA). The culture was maintained in a humidified chamber (5% carbon dioxide [CO_2], 37°C). The NSCs (3^{rd} – 8^{th}) were used in the study.

2.5. Cell Counting Kit-8 (CCK-8) assay

NSCs were digested into single-cell suspension by enzyme and cultured at a density of 1×10^4/well in 100 μL of medium into 96-well microplate for 24 h. Then, the cells were treated with different concentrations of SPIO (0, 25, 50, 100, 200, and 300 μg/mL). After treatment for 48 h, 10 μL of CCK-8 reagent was added to each well, and then cultured for 4 h. The absorbance was analyzed at 450 nm.

2.6. tMCAO

tMCAO was processed according to a previous study[12,21-23]. Briefly, anesthesia was induced with 3% of isoflurane, 30% of oxygen, and 70% of nitrous oxide in an anesthetic chamber and maintained with 1.5% of isoflurane through a facemask. A temperature-controlled heating pad was used to maintain the temperature at 37.0 ± 0.5°C during the surgery and recovery period. A 1 cm midline skin incision was made over the neck, and the right common carotid artery (CCA) was carefully dissected free from the surrounding nerves under a stereo dissecting microscope and tied off, exposing the right external carotid artery (ECA)

and isolating from its small artery branches. The ECA was ligated 3 mm distal to its origin, and an arteriotomy was then performed on the ECA. Following that, a silicone-rubber-coated nylon filament (602356PK5Re, Doccol Co., USA) was inserted into the ECA and advanced for 9 – 10 mm to the carotid bifurcation along the internal carotid artery (ICA) and the origin of the middle cerebral artery (MCA). The incision in the neck was subsequently sutured. After 1 h of occlusion, the silicone-rubber-coated nylon filament was removed to restore blood flow. In sham mice, the filament was not inserted.

Using the Moor full-field laser perfusion imager (moorFLPI-2, Moor Instruments, Axminster, UK), the cortical cerebral blood flow was measured in both cerebral hemispheres and recorded 15 min before MCAO and throughout the ischemic period until 15 min after the onset of reperfusion. Animals that did not show a reduction in cerebral blood flow (CBF) of at least 75% of the baseline level or died after ischemic induction (<10%) were excluded from further experimentation[24]. A total of 33 mice were included in this study; however, based on the aforementioned criteria, seven mice were excluded due to death (n = 4) or failed stroke induction (n = 3).

2.7. Microinjection of neural stem cells

The lateral ventricles of mice were microinjected with NSCs at 7 days after tMCAO at the following coordinates: 0.3 mm behind the bregma and 1.0 mm lateral from the sagittal midline, at a depth of 2.2 mm from the skull surface. A total of 2 μL of cell suspension (1×10^6 cells) was injected into the left lateral ventricle at a rate of 0.1 μL/min by microinjection pump.

2.8. Magnetic resonance imaging scanning

In vivo MRI was performed using a 7-Tesla small animal MRI scanner (BrukerPharmaScan, Karlsruhe, Germany). The mice were anesthetized with 2% of isoflurane delivered through a nose cone. The respiratory rate and body temperature of the mice were monitored through a physiology monitor. All sequences were performed on the murine cerebrum in axial/coronal orientation with the following parameters: Multigradient-echo sequences (12 echos; repetition time [TR], 1,500 ms; first echo at 4 ms; echo spacing 7 ms; field of view [FOV], 20 × 20 mm^2; matrix, 256 × 256; slice thickness, 0.6 mm, 8 average) were used for T2-star mapping.

2.9. Near-infrared fluorescence (NIF) imaging

The mice were microinjected with SPIO-labeled NSCs at 7 d after tMCAO. NIF images were obtained with the Maestro *In Vivo* Imaging System (Cri Inc., USA). Near-infrared light was collected with an excitation bandpass filter of 540–580 nm and a long-pass emission filter of >600 nm.

2.10. Western blot (WB) analysis

Proteins were extracted in a radioimmunoprecipitation assay (RIPA) lysis buffer (P0013B, Beyotime, China), separated on sodium dodecyl sulfate-polyacrylamide gels, and electrophoretically transferred onto polyvinylidene fluoride (PVDF) membranes according to our previously described methods[23]. The membranes were blocked with 5% of non-fat dry milk in TBST, probed with antibodies recognizing glial fibrillary acidic protein (GFAP, 16825-1-AP, 1:1000, Proteintech), Nestin (66259-1-Ig, 1:1000, Proteintech), TUJ1 (ab18207, 1:1000, Abcam), and β-actin (60008-1-Ig, 1:1000, Proteintech) overnight at 4℃ before incubation with a horseradish peroxidase-conjugated goat anti-mouse/rabbit immunoglobulin (Ig) G secondary antibody (SA00001-1/SA00001-2, 1:2000, Proteintech). To detect the signals, the Microchemi 4.2® (DNR Bio-Imaging Systems, Jerusalem, Israel) digital image scanner was used. Individual protein bands were quantified by densitometry using Image J.

2.11. Prussian blue staining

The brain tissue was cut into 30 μm-thick sections. The tissue sections were washed every 5 min with PBS (3 times). Prussian blue (PB) staining was performed using a Prussian Blue Staining Kit (Sigma-Aldrich) according to the user manual. In brief, tissue sections were incubated with Perls stain for 30 min at room temperature. Then, the tissue sections were washed with distilled water for 5 min. The sections were covered with counterstaining solution for 10 min and subsequently washed with distilled water for 5 min. The tissue sections were sealed with neutral resin.

2.12. Bromodeoxyurdine (BrdU) labeling

NSCs were prepared on poly-L-lysine-coated glass coverslips. 10 μL of Dulbecco's PBS containing 10 mM BrdU was added in the 1 mL cell culture medium. After 4 h, the cells were fixed overnight in 4% of paraformaldehyde (PFA) at 4°C. The cells were then denatured with HCl for 30 min at room temperature and washed with PBS 2 times. Subsequently, the cells were blocked for 1 h in PBS containing 10% normal goat serum and 0.1% Triton X-100 at room temperature. The blocked cells were incubated with mouse anti-BrdU antibody (1:200; SC-32323, Santa Cruz, CA) that were diluted in PBS containing 10% normal goat serum and 0.1% Triton X-100 at 4°C overnight. The samples were washed with PBS 3 times before being incubated with donkey anti-mouse (conjugated to Alexa-Fluor 576) secondary antibody for 1 h at room temperature. After washing with PBS for another 3 times, the samples were mounted using ProLong

Gold Antifade Reagent with 4',6-diamidino-2-phenylindole (DAPI) (0100-20, SouthernBiotech, USA). The samples were imaged by microscopy (Olympus, Japan) and analyzed by Image J software.

2.13. Immunofluorescence (IF) staining

Before staining, for tissue samples, coronal slices (30 μm) were prepared from brain tissues and incubated with Triton X-100 (0.25% in PBS) for 15 min, and subsequently blocked for 1 h in PBS containing 10% of normal goat serum and 0.1% of Triton X-100 at room temperature. For cell samples, the NSCs were fixed for 20 min in 4% of PFA in PBS and washed 3 times with PBS. Then, the cells were incubated with Triton X-100 (0.25% in PBS) for 15 min and blocked for 1 h in PBS containing 10% of normal goat serum and 0.1% of Triton X-100 at room temperature. The blocked sections and cells were incubated with primary antibodies that were diluted in PBS containing 10% normal goat serum and 0.1% Triton X-100 at 4°C overnight. The primary antibodies used in this study contained GFAP (16825-1-AP, Proteintech) and TUJ1 (ab18207, Abcam). The samples were then washes with PBS three times before being incubated with secondary antibodies, including Alexa Fluor 594 goat anti-mouse IgG (A-11005, Invitrogen) and Alexa Fluor 594 goat anti-rabbit IgG (A-11012, Invitrogen) for 1 h at room temperature. The samples were washed with PBS three times and mounted using ProLong Gold Antifade Reagent with DAPI (Southern Biotech, USA). The samples were imaged by microscopy (Olympus, Japan) and analyzed by Image J software.

2.14. Statistics

The data were presented as mean ± standard error of the mean (SEM). Significance was established using t-test for paired values. One-way analysis of variance (ANOVA) followed by Holm-Sidak *post hoc* test was used for comparisons of three groups. Statistical analysis was performed using GraphPad Prism 8.0.

3. Results

3.1. Characterization of SPIO -labeled neural stem cells

To determine the characterization of transplanted NSCs labeled with SPIO, we first evaluated the viability of SPIO-labeled NSCs. CCK-8 assay was performed with NSCs that were incubated with SPIO nanoparticles at concentrations of 0, 25, 50, 100, 200, and 300 μg/mL for 48 h. As shown in Figure 1A, SPIO nanoparticle concentrations of <300 μg/mL showed low cytotoxicity (90%). The viability decreased by 9.64% when the concentration was 300 μg/mL. We then explored the uptake of SPIO nanoparticles, in which a PB staining assay was performed. The results showed that the intake of SPIO by NSCs was directly proportional to the concentration of SPIO (Figure 1B). Subsequently, MRI was used to test the signal of SPIO nanoparticles at different concentrations (Figure 1C). With the increase in SPIO nanoparticle concentration, the MRI signal increased gradually, and markedly enhanced signals were observed in samples with concentration over 200 μg/mL. Moreover, we assessed the concentration-related internalization of SPIO by ICP-MS (Figure 1D). We found that the amount of iron increased along with the concentration of SPIO, demonstrating that NSCs internalized SPIO nanoparticles in a dose-dependent manner. As shown in Figure 1E and F, SPIO-labeled NSCs did not affect cell proliferation. In conclusion, at a concentration of 200 μg/mL, the uptake of SPIO nanoparticles was sufficient and did not have any noticeable influence on the viability of NSCs. Therefore, we used 200 μg/mL for all experiments.

3.2. Differentiation of SPIO-labeled neural stem cells

Biosafety is a crucial requirement for nanotracers to label stem cells. To investigate whether SPIO is involved in the regulation of NSC differentiation, NSCs were labeled with SPIO at a concentration of 200 μg/mL. WB analysis showed that the levels of GFAP (the astrocyte marker) and TUJ1 (the neuronal marker) increased during NSC differentiation, while the expression of Nestin (NSC marker) significantly decreased. However, there was no significant difference in the expression of Nestin, GFAP, and TUJ1 between the SPIO-labeled NSCs (SPIO) group compared with the no SPIO-labeled NSCs (control) group (Figure 2A and B). The detection of GFAP expression at 1 day, 7 days, and 14 days after NSC treatment by IF staining showed that there was no significant difference in GFAP expression between the SPIO-labeled group and the control group (Figure 2C and D). Meanwhile, as shown in Figure 2E and F, there was no significant difference in TUJ1 expression between the SPIO-labeled group and the control group, indicating that SPIO did not influence the differentiation of NSCs.

3.3. Tracking the signal of SPIO nanoparticles in a tMCAO stroke model

To investigate the migration of transplanted NSCs labeled with SPIO, MRI was used to detect signals in the coronal plane of mouse brains that were detected by MRI. Signals were detected over the injection site at 7 days and the ischemic area at 14 days, 21 days, and 28 days in the SPIO-labeled NSCs group after tMCAO (Figure 3A). However, no signals of SPIO nanoparticles were detected in the control group. This suggests that NSCs labeled with SPIO nanoparticles could migrate from the injection site to the ischemic area. Besides, signals were also detected in the horizontal plane of mouse brains by MRI (Figure 3B).

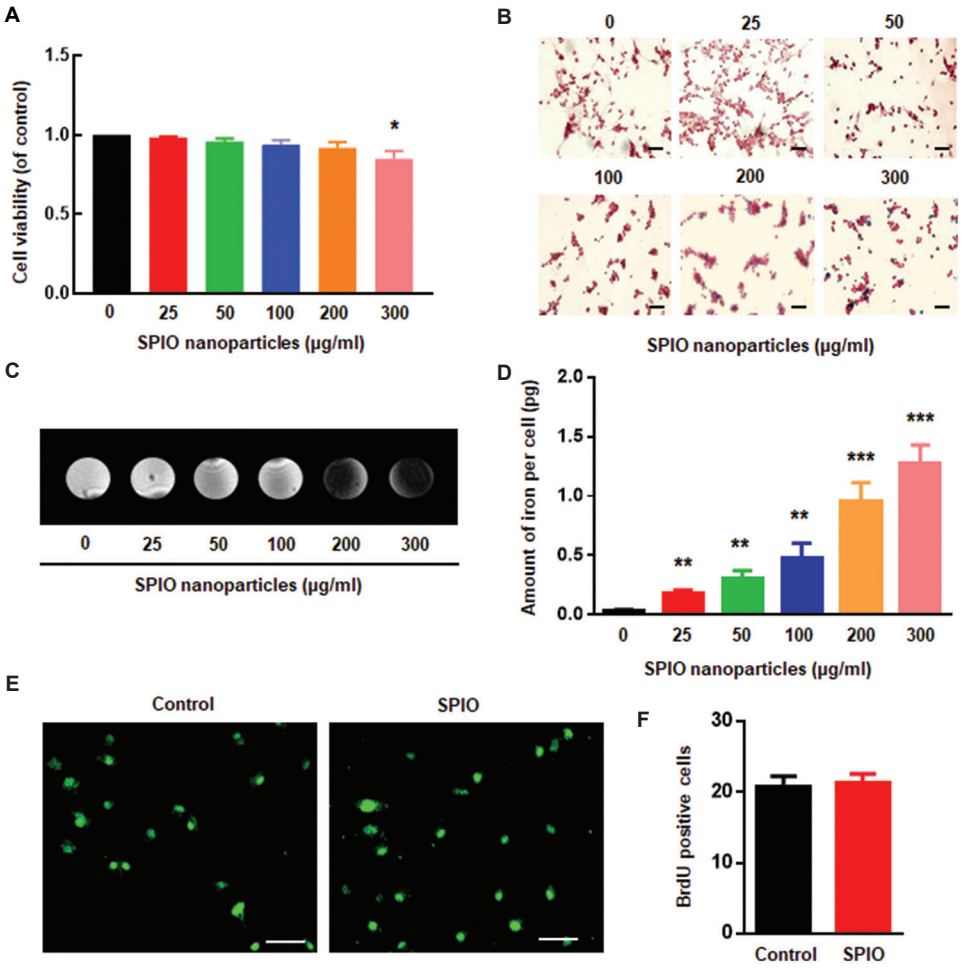


Figure 1. Characterization of SPIO-labeled neural stem cells (NSCs). (A–D) NSCs were incubated with SPIO at six different concentrations. (A) Cell viability of NSCs treated with SPIO at various concentrations. Cell viability was measured by CCK-8 assay at 48 h after SPIO treatment. $n = 3$ independent experiments/group. (B) PB staining of NSCs treated with SPIO at different concentrations. NSCs were stained with PB iron at 48 h after SPIO treatment. Scale bar = 100 μm. (C) T2-weighted MRI analysis of NSCs at 48 h after SPIO treatment at different concentrations. (D) Total amounts of iron in NSCs treated with different concentrations of SPIOs for 48 h measured by ICP-MS. (E and F) BrdU labeling analysis of SPIO-labeled NSCs. (E) Representative images. Scale bar = 100 μm. (F) Quantitative analysis. All data are presented as mean ± SEM of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus the control group.

BrdU: Bromodeoxyuridine: ICP-MS: Inductively coupled plasma mass spectrometer, MRI: Magnetic resonance imaging, NSCs: Neural stem cells, PB: Prussian blue, SEM: Standard error of the mean, SPIO: Superparamagnetic iron oxide.

On the other hand, PB staining can also be used for tracking transplanted NSCs in tMCAO mice. As shown in Figure 3C, SPIO-labeled NSCs were identified over the injection site and infarcted area at 28 days after tMCAO. These results suggest that SPIO labeling allows *in vivo* tracking of transplanted NSCs with MRI. The results also confirmed the migration of transplanted NSCs from the injection site to the infarcted area in tMCAO mouse brain.

3.4. Transplanted neural stem cells labeled with SPIO in tMCAO mice

To confirm whether the signals of SPIO nanoparticles detected by MRI and PB staining in the ischemic area are carried by NSCs, we examined the correlation between transplanted NSCs and SPIO nanoparticles. The synthesis of SPIO nanoparticles labeled with rhodamine B (RhB) has been described in previous research[25]. As shown in Figure 4A, the GFP^+ NSCs labeling rate (green) was colocalized with RhB-labeled SPIO (red) *in vitro*. Microinjection of SPIO-RhB-labeled GFP^+ NSCs was then performed in tMCAO mice. At 28 days after tMCAO, a fluorescence signal was detected in the peri-infarct area of the cortex in the ischemic hemisphere (Figure 4B). This result demonstrated that the SPIO-labeled NSCs had migrated to the ischemic area. The above experiments not only confirmed that the transplanted NSCs labeled with

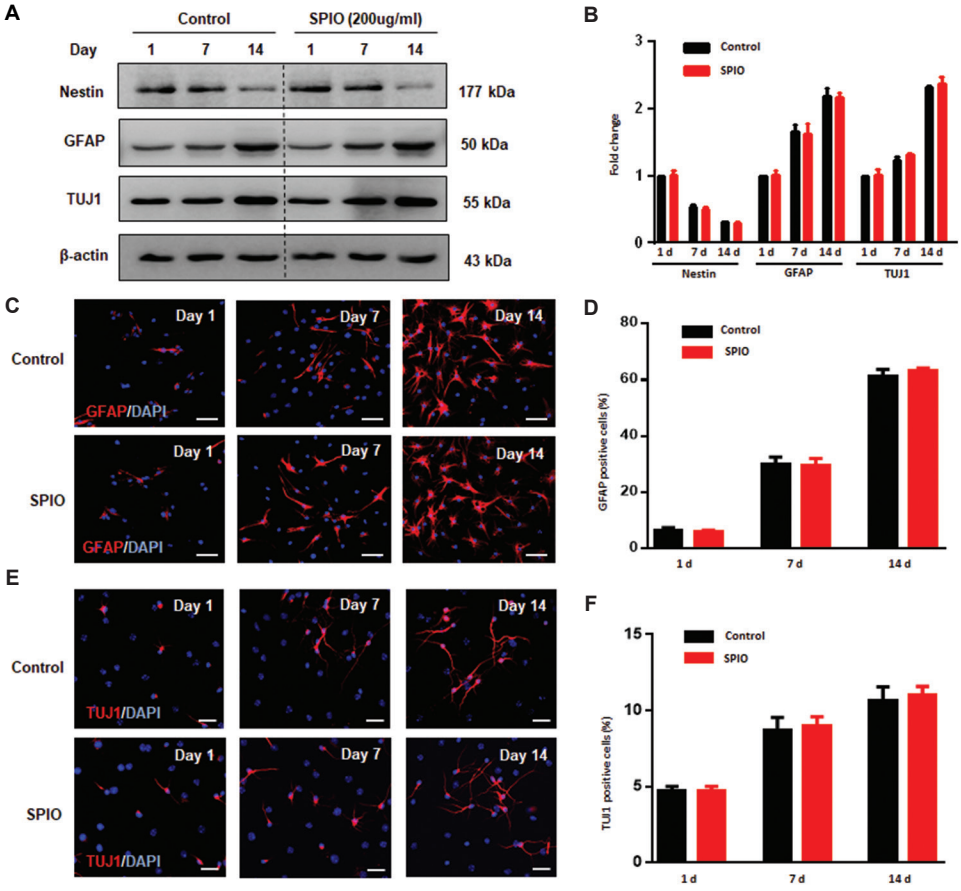


Figure 2. Differentiation of SPIO-labeled NSCs. (A and B) Western blot analysis of Nestin, GFAP, and TUJ1 expression in SPIO-labeled NSCs and control-NSCs. Timescale indicates days after NSC differentiation. (C–F) Immunofluorescence staining of GFAP and TUJ1 in SPIO-labeled NSCs and control-NSCs. NSCs labeled with or without SPIO were cultured in differentiation medium for 1, 7, and 14 days, and then stained with cells positive for GFAP and TUJ1. GFAP and TUJ1 were stained in red and DAPI was stained in blue. Scale bar = 50 μm. Histogram showing the number of GFAP and TUJ1-positive cells in NSCs labeled with or without SPIO and cultured for 1, 7, and 14 d in differentiation medium. Timescale indicates days after NSCs differentiation. Quantification of GFAP and TUJ1 immunofluorescence-positive cell numbers using ImageJ software. Data represent the mean of at least three independent experiments ± SEM.
GFAP: Glial fibrillary acidic protein, control-NSCs: Control, NSCs: Neural stem cells, SEM: Standard error of the mean, SPIO: Superparamagnetic iron oxide, SPIO-labeled NSCs: SPIO, TUJ: β-tubulin III.

SPIO migrated to the infarcted side, but also verified that the signals of SPIO nanoparticles detected by MRI and PB are carried by NSCs.

4. Discussion

In recent years, stem cell therapy has become a very promising and advanced scientific research method[26] that can be used for various diseases, such as stroke[27], Parkinson's disease[28], amyotrophic lateral sclerosis[29], cancer[30], and heart disease[31]. Among them, for stroke, stem cell therapy shows promising prospects as it offers potential neurorestorative benefits. The potential of NSCs for cell therapy is enormous. Transplanted NSCs have been used in pre-clinical and clinical trials to restore function[32]. However, some relevant problems need to be addressed, especially pertaining the persistence and differentiation of transplanted cells into brain tissue.

With regard to this issue, we developed a method that allows us to observe the fate of transplanted cells *in vivo*, that is, to track stem cells and monitor stem cell homing, migration, and proliferation to evaluate the therapeutic effect.

In the present work, we developed a novel cell labeling strategy based on SPIO nanoparticles and explored its application for long-term MRI imaging tracking of transplanted NSCs. The SPIO nanoparticles showed a narrow size distribution, superparamagnetism, and low cytotoxicity. Our result demonstrated the tracking ability of SPIO nanoparticles. However, the biosafety of SPIO nanoparticles should be evaluated before further clinical application. In

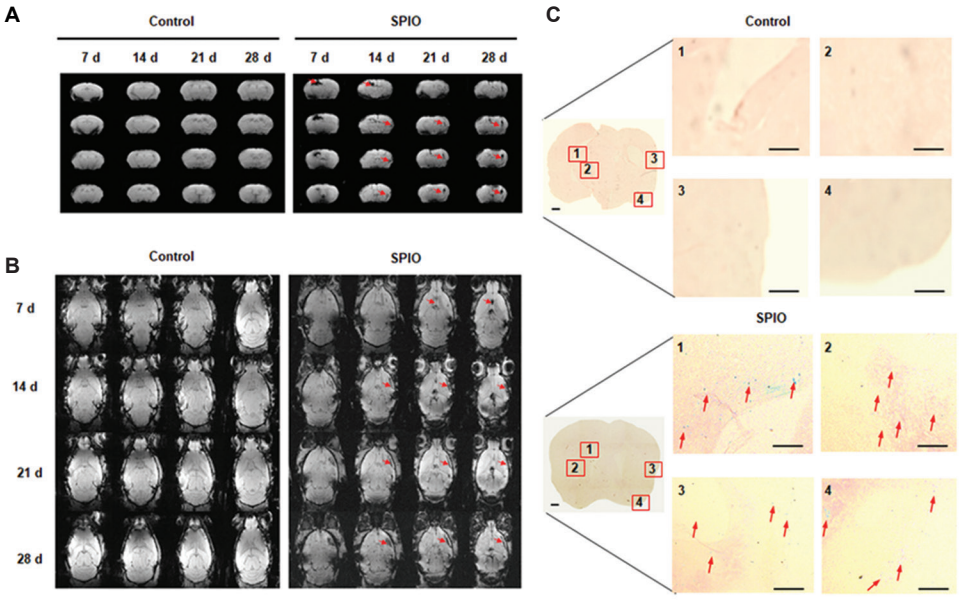


Figure 3. Tracing of transplanted NSCs labeled with SPIO in tMCAO mice. (A) Representative images of MRI T2-star coronal plane showing the migration and distribution of transplanted NSCs. MRI was taken at 7, 14, 21, and 28 days after tMCAO surgery. $n = 3$ in each group. (B) Representative images of MRI T2-star horizontal plane showing the migration and distribution of transplanted NSCs. MRI was taken at 7, 14, 21, and 28 d after tMCAO surgery. $n = 3$ in each group. (C) Representative images of PB staining showing the distribution of iron deposits in the brain at 28 days after tMCAO surgery. Insets, individual images from PB staining. Red arrows point to SPIO signals. Scale bar = 100 μm.
MRI: Magnetic resonance imaging, NSCs: Neural stem cells, SPIO: Superparamagnetic iron oxide, tMCAO: transient middle cerebral artery occlusion.

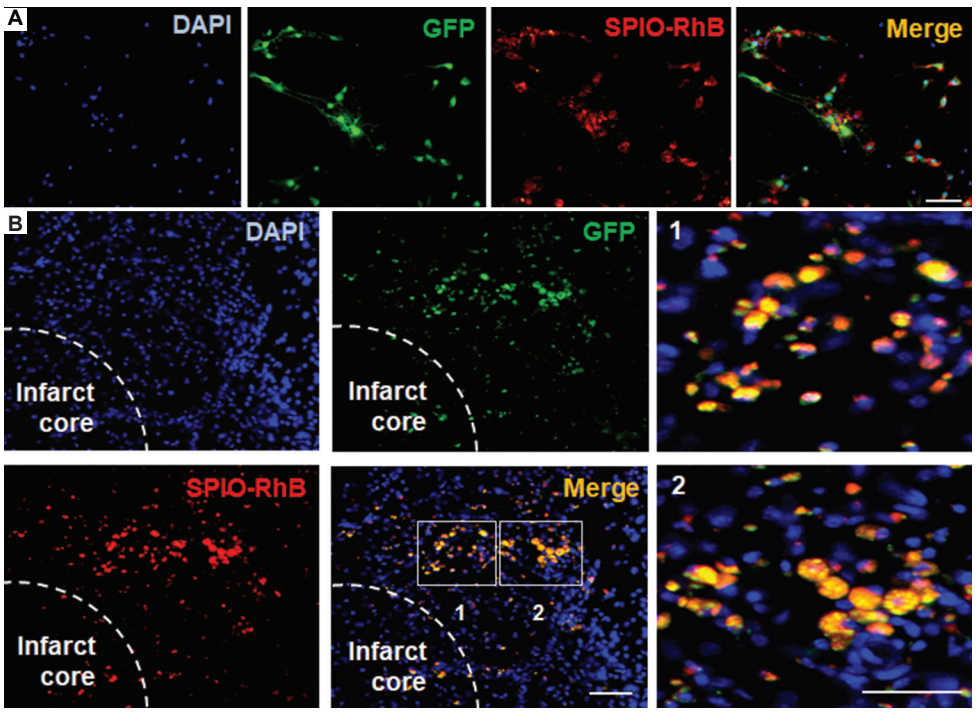


Figure 4. Tracing transplanted NSCs labeled with SPIO in tMCAO mice. (A) Verification of NSCs labeling rate of synthetic rhodamine B (Rhd B)-SPIOs *in vitro*. Scale bar = 50 μm. (B) Immunofluorescence evaluation of peri-infarct cortex implanted with SPIO-labeled NSCs. DAPI (blue), nucleus; GFP (green), NSCs; SPIO-Rhd-B (red), which were coated with dextran and visualized with a rhodamine-conjugated dextran. Scale bar = 100 μm.
DAPI: 4',6-diamidino-2-phenylindole, GFP: Green fluorescent protein, NSCs: Neural stem cells, PB: Prussian blue, Rhd B: Rhodamine B, SPIO: Superparamagnetic iron oxide, tMCAO: transient middle cerebral artery occlusion.

this study, we first identified the appropriate concentration of SPIO nanoparticles. The results showed that the amount of iron increased with the increase of SPIO concentration, demonstrating that NSCs internalized SPIO nanoparticles in a dose-dependent manner; thus, we chose an SPIO nanoparticle concentration of 200 μg/mL. This SPIO concentration had no significant effect on the proliferation and differentiation of NSCs, providing high biological security to track NSCs in the tMCAO mice model. Developing long-term tracking is a major problem in stem cell therapy. A marker should first effectively bind to the stem cell and the signal should not disappear within a short time[33,34]. In this study, SPIO nanoparticles were used to label NSCs and were tracked by MRI at different time points, demonstrating that transplanted SPIO-labeled NSCs can migrate to infarcted areas. Taken together, the current work based on SPIO-based MRI imaging enables the visualization of the distribution, migration, and survival of transplanted NSCs in tMCAO mice, thereby facilitating a good understanding of the role of NSCs in ischemic stroke.

5. Conclusion

In this study, we demonstrated that SPIO nanoparticles could be used for tracking NSCs in a mice tMCAO stroke model by MRI. Our result showed that the uptake of SPIO nanoparticles by NSCs is concentration dependent, in which better magnetic properties were observed with higher concentrations of SPIO nanoparticles than lower concentrations. Moreover, our result showed that transplanted NSCs labeled with SPIO can migrate from the injection site to the infarcted area *in vivo*, thus providing the salient point for stroke treatment with NSCs. We conclude that SPIO nanoparticles can be employed to track the migration of NSCs, thereby advancing stem cell-based therapy toward clinical application.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Author contributions

Conceptualization: Ying Bai and Bing Han
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Writing – original draft: Ying Bai and Guangtian Wang
Writing – review & editing: Guangtian Wang and Bing Han

Ethics approval and consent to participate

All animal experiments were performed in accordance with the standard guidelines for the care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee of the Medical School of Southeast University.

Consent for publication

Not applicable.

Availability of data

Data can be obtained from the corresponding author following request.

References

1. Datta A, Sarmah D, Mounica L, *et al.*, 2020, Cell death pathways in ischemic stroke and targeted pharmacotherapy. *Transl Stroke Res*, 11(6): 1185–1202.

   https://doi.org/10.1007/s12975-020-00806-z

2. Herpich F, Rincon F, 2020, Management of acute ischemic stroke. *Crit Care Med*, 48(11): 1654–1663.

   https://doi.org/10.1097/CCM.0000000000004597

3. Tao T, Liu M, Chen M, *et al.*, 2020, Natural medicine in neuroprotection for ischemic stroke: Challenges and prospective. *Pharmacol Ther*, 216: 107695.

   https://doi.org/10.1016/j.pharmthera.2020.107695

4. Wang F, Tang H, Zhu J, *et al.*, 2018, Transplanting mesenchymal stem cells for treatment of ischemic stroke. *Cell Transplant*, 27(12): 1825–1834.

   https://doi.org/10.1177/0963689718795424

5. Jiang XC, Xiang JJ, Wu HH, *et al.*, 2019, Neural stem cells transfected with reactive oxygen species-responsive polyplexes for effective treatment of ischemic stroke. *Adv Mater*, 31(10): e1807591.

   https://doi.org/10.1002/adma.201807591

6. Wechsler LR, Bates D, Stroemer P, *et al.*, 2018, Cell therapy for chronic stroke. *Stroke*, 49(5): 1066–1074.

   https://doi.org/10.1161/STROKEAHA.117.018290

7. Bai Y, Ren H, Bian L, *et al.*, 2022, Regulation of glial function by noncoding RNA in central nervous system disease. *Neurosci Bull*.

   https://doi.org/10.1007/s12264-022-00950-6

8. Chen J, Venkat P, Zacharek A, *et al.*, 2014, Neurorestorative therapy for stroke. *Front Hum Neurosci*, 8: 382.

https://doi.org/10.3389/fnhum.2014.00382

9. Hamblin MH, Lee JP, 2021, Neural stem cells for early ischemic stroke. *Int J Mol Sci*, 22(14): 7703.

https://doi.org/10.3390/ijms22147703

10. Ding DC, Lin CH, Shyu WC, *et al.*, 2013, Neural stem cells and stroke. *Cell Transplant*, 22(4): 619–630.

https://doi.org/10.3727/096368912X655091

11. Bernstock JD, Peruzzotti-Jametti L, Ye D, *et al.*, 2017, Neural stem cell transplantation in ischemic stroke: A role for preconditioning and cellular engineering. *J Cereb Blood Flow Metab*, 37(7): 2314–2319.

https://doi.org/10.1177/0271678X17700432

12. Wang G, Han B, Shen L, *et al.*, 2020, Silencing of circular RNA HIPK2 in neural stem cells enhances functional recovery following ischaemic stroke. *EBioMedicine*, 52: 102660.

https://doi.org/10.1016/j.ebiom.2020.102660

13. Tong L, Zhao M, Zhu S, *et al.*, 2011, Synthesis and application of superparamagnetic iron oxide nanoparticles in targeted therapy and imaging of cancer. *Front Med*, 5(4): 379–387.

https://doi.org/10.1007/s11684-011-0162-6

14. Kim SJ, Lewis B, Steiner MS, *et al.*, 2016, Superparamagnetic iron oxide nanoparticles for direct labeling of stem cells and *in vivo* MRI tracking. *Contrast Media Mol Imaging*, 11(1): 55–64.

https://doi.org/10.1002/cmmi.1658

15. Boese AC, Le QS, Pham D, *et al.*, 2018, Neural stem cell therapy for subacute and chronic ischemic stroke. *Stem Cell Res Ther*, 9(1): 154.

https://doi.org/10.1186/s13287-018-0913-2

16. Wang P, Ma S, Ning G, *et al.*, 2020, Entry-prohibited effect of kHz pulsed magnetic field upon interaction between SPIO nanoparticles and mesenchymal stem cells. *IEEE Trans Biomed Eng*, 67(4): 1152–1158.

https://doi.org/10.1109/TBME.2019.2931774

17. Keselman P, Yu EY, Zhou XY, *et al.*, 2017, Tracking short-term biodistribution and long-term clearance of SPIO tracers in magnetic particle imaging. *Phys Med Biol*, 62(9): 3440–3453.

https://doi.org/10.1088/1361-6560/aa5f48

18. Bashir MR, Bhatti L, Marin D, *et al.*, 2015, Emerging applications for ferumoxytol as a contrast agent in MRI. *J Magn Reson Imaging*, 41(4): 884–898.

https://doi.org/10.1002/jmri.24691

19. Chen B, Sun J, Fan F, *et al.*, 2018, Ferumoxytol of ultrahigh magnetization produced by hydrocooling and magnetically internal heating co-precipitation. *Nanoscale*, 10(16): 7369–7376.

https://doi.org/10.1039/c8nr00736e

20. Hu Y, Li D, Wei H, *et al.*, 2021, Neurite extension and orientation of spiral ganglion neurons can be directed by superparamagnetic iron oxide nanoparticles in a magnetic field. *Int J Nanomedicine*, 16: 4515–4526.

https://doi.org/10.2147/IJN.S313673

21. Wang Z, Xu P, Chen B, *et al.*, 2018, Identifying circRNA-associated-ceRNA networks in the hippocampus of Abeta1-42-induced Alzheimer's disease-like rats using microarray analysis. *Aging (Albany NY)*, 10(4): 775–788.

https://doi.org/10.18632/aging.101427

22. Wu F, Han B, Wu S, *et al.*, 2019, Circular RNA TLK1 aggravates neuronal injury and neurological deficits after ischemic stroke via miR-335-3p/TIPARP. *J Neurosci*, 39(37): 7369–7393.

https://doi.org/10.1523/JNEUROSCI.0299-19.2019

23. Bai Y, Zhang Y, Han B, *et al.*, 2018, Circular RNA DLGAP4 ameliorates ischemic stroke outcomes by targeting miR-143 to regulate endothelial-mesenchymal transition associated with blood-brain barrier integrity. *J Neurosci*, 38(1): 32–50.

https://doi.org/10.1523/JNEUROSCI.1348-17.2017

24. Shi Y, Zhang L, Pu H, *et al.*, 2016, Rapid endothelial cytoskeletal reorganization enables early blood-brain barrier disruption and long-term ischaemic reperfusion brain injury. *Nat Commun*, 7: 10523.

https://doi.org/10.1038/ncomms10523

25. Zhao J, Zhang Z, Xue Y, *et al.*, 2018, Anti-tumor macrophages activated by ferumoxytol combined or surface-functionalized with the TLR3 agonist poly (I: C) promote melanoma regression. *Theranostics*, 8(22): 6307–6321.

https://doi.org/10.7150/thno.29746

26. Zakrzewski W, Dobrzynski M, Szymonowicz M, *et al.*, 2019, Stem cells: Past, present, and future. *Stem Cell Res Ther*, 10(1): 68.

https://doi.org/10.1186/s13287-019-1165-5

27. Kim HY, Kim TJ, Kang L, *et al.*, 2020, Mesenchymal stem cell-derived magnetic extracellular nanovesicles for targeting and treatment of ischemic stroke. *Biomaterials*, 243: 119942.

https://doi.org/10.1016/j.biomaterials.2020.119942

28. Sonntag KC, Song B, Lee N, *et al.*, 2018, Pluripotent stem cell-based therapy for Parkinson's disease: Current status and future prospects. *Prog Neurobiol*, 168: 1–20.

https://doi.org/10.1016/j.pneurobio.2018.04.005

29. Okano H, Yasuda D, Fujimori K, *et al.*, 2020, Ropinirole, a new ALS drug candidate developed using iPSCs. *Trends Pharmacol Sci*, 41(2): 99–109.

https://doi.org/10.1016/j.tips.2019.12.002

30. Reya T, Morrison SJ, Clarke MF, *et al*., 2001, Stem cells, cancer, and cancer stem cells. *Nature*, 414(6859): 105–111.

https://doi.org/10.1038/35102167

31. Rikhtegar R, Pezeshkian M, Dolati S, *et al*., 2019, Stem cells as therapy for heart disease: iPSCs, ESCs, CSCs, and skeletal myoblasts. *Biomed Pharmacother*, 109: 304–313.

https://doi.org/10.1016/j.biopha.2018.10.065

32. Chrostek MR, Fellows EG, Crane AT, *et al*., 2019, Efficacy of stem cell-based therapies for stroke. *Brain Res*, 1722: 146362.

https://doi.org/10.1016/j.brainres.2019.146362

33. Hong H, Yang Y, Zhang Y, *et al*., 2010, Non-invasive cell tracking in cancer and cancer therapy. *Curr Top Med Chem*, 10(12): 1237–1248.

https://doi.org/10.2174/156802610791384234

34. Kircher MF, Gambhir SS, Grimm J, 2011, Noninvasive cell-tracking methods. *Nat Rev Clin Oncol*, 8(11): 677–688.

https://doi.org/10.1038/nrclinonc.2011.141

ACCSCIENCE PUBLISHING

Advanced Neurology

ORIGINAL RESEARCH ARTICLE

Emx1-Cre-mediated inactivation of PDK1 prevents plaque deposition in an Alzheimer's disease-like mouse model

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Abstract

BX912, an inhibitor for 3-phosphoinositide-dependent protein kinase 1 (PDK1), has been shown to produce beneficial effects in mouse models of Alzheimer's disease (AD). To test the hypothesis that early inhibition on PDK1 may prevent neuropathology in a 5×FAD mouse model, we employed a genetic approach to generate a mutant line, termed as *Pdk1* cKO/5×FAD, in which PDK1 is inactivated, specifically in the developing cortex of 5×FAD mice through *Emx1-Cre*-mediated gene recombination. We discovered that the *Pdk1* cKO/5×FAD mice exhibited a massive reduction of plaque pathology compared with their 5×FAD littermates. We also demonstrated that gliosis was remarkably attenuated in *Pdk1* cKO/5×FAD cortices and amyloid precursor protein levels were significantly lower in *Pdk1* cKO/5×FAD cortices compared with 5×FAD littermates. This study suggests that early inhibition on PDK1 may effectively prevent AD-like neuropathology.

Keywords: Alzheimer's disease; Plaque deposition; PDK1; Neuroinflammation; Amyloid precursor protein; Conditional knockout

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1. Introduction

Alzheimer's disease (AD) is the most common type of dementia in the elderly. The typical pathological hallmarks include extracellular amyloid β-protein (Aβ) deposition, intracellular tangles formed by phosphorylated tau, and gliosis[1-3]. The mechanisms underlying AD pathogenesis remain to be determined. A number of factors, including Aβ[4,5] and tau[6], have been identified as important factors for the etiology of AD. It is believed that Aβ accumulation may induce inflammation and cause neuronal death in the brain[5]. Many studies have demonstrated that Aβ is a major target for the treatment of AD[7-12].

3-phosphoinositide-dependent protein kinase 1 (PDK1) is a well-known member in the phosphoinositide 3 kinase (PI3K) pathway. The PI3K signaling pathway has important physiological roles, including cell proliferation, cell metabolism, angiogenesis, and so on[13-21]. Evidence has suggested that PDK1 may be involved in AD. First, it has been shown that AD patients exhibit enhanced PDK1 activity in the brain compared with control subjects without AD[22,23]. Second, a recent transcriptomic study demonstrated a significant increase in *PDK1* mRNA levels in excitatory neurons in the prefrontal cortex of AD patients compared with control subjects[24]. Third, it has been found that PDK1 activity is enhanced in several lines of amyloid precursor protein (APP) transgenic (Tg) mice, including Tg2576, APP/presenilin-1 (PS1), and 3×Tg-AD mice[22,25,26]. Moreover, previous evidence has shown that BX912, a potent PDK1 inhibitor, significantly diminishes amyloid plaques and improves spatial memory in mouse models of AD[22], raising a possibility that PDK1 may serve as a therapeutic target for AD.

Pietri *et al.* explored the mechanisms underlying BX912-induced beneficial effects in mouse models of AD. They found that BX912 increases the activity of α-secretase through tumor necrosis factor-α-converting enzyme (TACE), promotes the trans-localization of TACE into the plasma membrane, restores α-secretase-dependent cleavage of APP, and attenuates Aβ-induced neurotoxicity[22]. The study suggests that the inhibition of PDK1 may be effective in halting AD. However, serious side effects have been reported from the inhibition of PDK1 pharmacologically or genetically. First, long-term treatment with BX912 results in the death of adult APP Tg mice[22]. Second, a straight knockout (KO) of PDK1 causes embryonic lethality in mice[27]. Third, neural progenitor cell (NPC)-specific inactivation of PDK1 results in apoptosis of cortical neurons and impairs spatial learning in mice[28]. Fourth, interneuron- or oligodendrocyte-specific deletion of PDK1 or its substrate Akt leads to postnatal death in mice before the age of day 21 (P21)[15,16,29].

In response to the findings of Pietri *et al.*, we propose that early inhibition of PDK1 function may prevent AD-like pathology in APP Tg mice. To test this hypothesis, we employed a genetic strategy to inactivate PDK1 in the developing cortices of 5×FAD mice. The latter, which have been widely used in the field of AD, are known to express five mutations on human *APP* and *PS1* genes[30]. It has been reported that plaque pathology begins to become apparent in the cortex and the subiculum in 5×FAD mice at 2 months[30]. On the other hand, Cre is known to be expressed mainly in NPCs in the developing cortex and in excitatory neurons in the postnatal forebrain of *Emx1-Cre* mice[31-34]. In this study, *Emx1-Cre* mice were bred with 5×FAD (*APP/PS1*)[30,35,36] and floxed *Pdk1*$^{f/f}$[28,37-39] to generate *Pdk1*$^{f/f}$*;Emx1-Cre;APP/PS1* mice, termed as *Pdk1* cKO/5×FAD hereafter. Amyloid plaques were hardly detected in *Pdk1* cKO/5×FAD cortices, and the population of ionized calcium-binding adaptor molecule 1 (Iba1)+ cells decreased significantly. Furthermore, there was a significant decrease in APP levels in *Pdk1* cKO/5×FAD mice compared with their 5×FAD littermates. The above findings support the notion that early inhibition of PDK1 may effectively prevent AD-like pathology.

2. Materials and methods

2.1. Mice

The mouse work was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Nanjing University. The background strain for male and female mice was C57BL/6. All mouse lines, including *Pdk1*$^{f/f}$, *Emx1-Cre*, and 5×FAD mice, are described in our previous studies[15,28,33,35,37]. The mice were maintained in an animal facility at the Model Animal Research Center of Nanjing University. They were group-housed in a room without specific pathogens. The room temperature was maintained at 25 ± 1°C, and the mice had unrestricted access to food and water. The animal protocol (AP) for this study was approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing University.

The following primers were used for genotyping: Primers for floxed *Pdk1* were TGTGCTTGGTGGATATTGAT (forward) and AAGGAGGAGAGGAGGAATGT (reverse); primers for human *APP* were AGGACTGACCACTCGACCAG (forward) and CGGGGGTCTAGTTCTGCAT (reverse); primers for human *PS1* were AATAGA GAACGGCAGGAGCA (forward) and GCCATGAGGGCACTAATCAT (reverse); and primers for *Cre* were GCCTGCATTACCGGTCGATGCAACGA (forward) and GTGGCAGATGGCGCGGCAACACCATT (reverse).

2.2. Tissue preparation for biochemical and morphological studies

The mice were perfused with cold phosphate-buffered solution (PBS)[40]. The brain was cut sagittally into two hemispheres, in which the cortex was dissected from one hemisphere to prepare protein samples. The other hemisphere was fixed in 4% paraformaldehyde (PFA) (Aladdin Biochemical Technology, C104188) at 4°C overnight and then dehydrated using a series of ethanol solutions (Nanjing Reagent, C0691510225). The dehydrated brain hemispheres were embedded in a paraffin machine (Leica Biosystems, 39601095) for the preparation

of paraffin blocks. There were four brain hemispheres in each paraffin block from four different genotype groups, including the control, *Pdk1* cKO, 5×FAD, and *Pdk1* cKO/5×FAD.

2.3. Immunohistochemistry (IHC)

We chose two brain sections, spaced at 400 μm, from each mouse for IHC. The sections were deparaffinized and then rehydrated with degraded ethanol solutions. They were treated with boiled sodium citrate buffer (Xilong Scientific, 160012) for 25 min. The sections were cooled down at room temperature and then blocked with 3% hydrogen peroxide (Sinopharm Chemical Reagent, 10011218) for 20 min. They were subsequently treated with 5% BSA solution (bovine serum albumin dissolved in PBS) (Best Biological, BA0029) for 20 min.

The sections were incubated with primary antibodies at 4°C overnight and then washed out with PBS 3 times before incubation with a secondary antibody at 4°C for 1 h. The DAB Kit (Vector Laboratories, SK-4100) was then used for the processing of the sections. After dehydration, each section was sealed using neutral resin.

All the primary antibodies are listed in Table 1.

For fluorescence IHC, the following secondary antibodies were obtained from Jackson ImmunoResearch Laboratories: Alexa Fluor 488 (715-545-150) and Alexa Fluor 594 (711-585-152). Fluorescence images and IHC images were captured using a confocal microscope from Leica (SP5) and an Olympus microscope (BX53), respectively.

2.4. Western blotting

Cortical tissues were homogenized using cold radioimmunoprecipitation assay (RIPA) buffer to prepare cortical protein lysates[41,42]. The gradients were as follows (in mM): 20 mM tris-hydrochloride (HCl), pH 7.4, 150 mM sodium chloride (NaCl), 1 mM ethylenediaminetetraacetic acid (EDTA), 1% NP-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (SDS, Yifeixue Biotechnology, YS0005-500). The RIPA lysis buffer contained protease and phosphatase inhibitors. Protein concentration was obtained using a method reported recently[43,44].

Cortical samples were normalized to determine the loading volume for each sample (total proteins = 40 μg). Samples were loaded into 10% SDS-PAGE running gel. Nitrocellulose membranes were used for protein transfer. After the membranes were blocked using a non-fat milk solution (Sangon Biotech, A600669) (5% w/v) for 1 h, they were then probed using the primary antibodies listed in Table 1 at 4°C overnight. The membranes were washed with PBS 3 times (15 min each time) and incubated with the following secondary antibodies: IRdye800 and IRdye680[45,46]. The membranes were scanned using the LI-COR Imaging System.

Table 1. Primary antibodies used for Western blotting and immunohistochemistry.

Antibodies	Suppliers	Catalogue number
Anti-PDK1 antibody	Abcam	ab52893 RRID: AB_881962
Anti-NeuN	Millipore	ABN78 RRID: AB_10807945
Anti-Aβ (1-16) antibody (6E10)	BioLegend	803001 RRID: AB_2564653
Anti-Aβ antibody (D54D2)	Cell Signaling Technology	8243 RRID: AB_2797642
Anti-amyloid precursor protein antibody	Sigma-Aldrich	A8717 RRID: AB_258409
Anti-GFAP antibody	ABclonal	A14673 RRID: AB_2761548
Anti-Iba1 antibody	FUJIFILM Wako Shibayagi	016-20001 RRID: AB_839506
Aniti-phospho-S6 (Ser235/236) antibody	Cell Signaling Technology	2211 RRID: AB_331679
Anti-phospho-4E-BP1 (Ser65) antibody	Cell Signaling Technology	9451 RRID: AB_330947
Anti-BACE1 antibody	Abcam	Ab183612 RRID: N/A
Anti-ADAM10 antibody	Millipore	AB19026 RRID: AB_2242320
Anti-β-actin antibody	GeneTex	4060 RRID: AB_2315049
Anti-GAPDH antibody	CW Biotech	cw0100 RRID: N/A

2.5. Counting methods for cells positive for different markers

For each mouse, we chose two brain sections (spaced 400 μm) to perform IHC for Aβ, glial fibrillary acidic protein (GFAP), or Iba1[37]. First, IHC images for Aβ were taken with the ×10 objective lens in the BX53 microscope, and amyloid plaques (diameter of plaques >5 μm) were counted in each IHC image (an area of 1.364 μm × 1.021 μm). Second, the same microscopy settings were used to capture IHC images for GFAP and Iba1. In each IHC image, GFAP+ cells or Iba1+ cells in the cortex were counted manually.

2.6. Statistical analysis

Data were shown as average ± standard error of mean (SEM). Two-tailed Student's t-test was used to analyze the differences in protein levels, cell numbers, and plaque

numbers between *Pdk1* cKO/5×FAD mice and their 5×FAD littermates. $P < 0.05$ indicated a significant effect.

3. Results

3.1. Molecular analysis of the 5×FAD model with PDK1 deficiency through *Emx1-Cre*-mediated gene recombination

It has been shown that long-term treatment of adult Tg2576 mice with BX912 decreases amyloid plaques but causes lethal effect in mice[22]. To investigate whether the inactivation of PDK1, beginning from the embryonic stage, could prevent AD-like pathology in 5×FAD mice[30], we generated *Pdk1*$^{f/f}$;*Emx1-Cre*/5×FAD (*Pdk1* cKO/5×FAD) mice, in which the deletion of PDK1 begins at embryonic day 10.5 (E10.5) in the forebrain neurons. The four different genotypes are shown in Figure 1A.

To determine the expression pattern of Cre, *Rosa26-LSL-tdTomato* mice[16,47] were crossed to *Emx1-Cre* to generate *Emx1-Cre;Rosa26-LSL-tdTomato*, in which tdTomato is expressed in Cre-positive (Cre+) cells. The brain sections of the double mutant mice at P0 were used to perform costaining for tdTomato and NeuN. We observed abundant tdTomato+/NeuN+ cells in the cortex (Figure 1B). The cell counting results showed that ~92% of NeuN+ cells were positive for tdTomato. Subsequently, we performed fluorescence IHC for PDK1. We found that PDK1 immunoreactivity was qualitatively reduced in the *Pdk1* cKO/5×FAD cortex at 2.5 months compared with 5×FAD littermates (Figure 1C). Western blotting was performed for PDK1 using cortical lysates from mice at 2.5 months. PDK1 levels were notably decreased in the *Pdk1* cKO/5×FAD cortex compared with 5×FAD mice (Figure 1D), suggesting efficient inactivation of PDK1 in *Pdk1* cKO/5×FAD mice.

3.2. Plaque deposition is prevented in 5×FAD mice with PDK1 deficiency

To investigate whether PDK1 deletion affects plaque deposition in 5×FAD mice, IHC was performed for Aβ using brain sections from control, *Pdk1* cKO, 5×FAD, and *Pdk1* cKO/5×FAD mice at three different ages (2.5, 4, and 6 months). First, there were a few amyloid plaques in the 5×FAD cortex at 2.5 months but abundant amyloid plaques at 4 and 6 months compared with non-Tg littermate controls (Figure 2A). In contrast, amyloid plaques were hardly seen in the *Pdk1* cKO/5×FAD cortex at the aforementioned ages

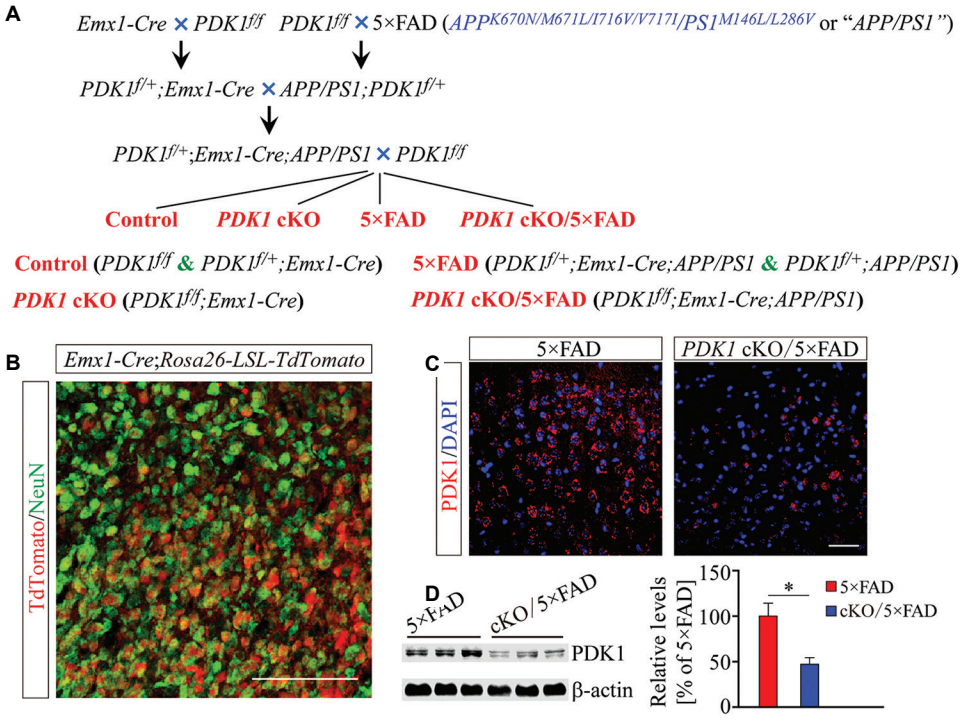


Figure 1. Characterization of forebrain-specific *Pdk1* cKO/5×FAD mice. **(A)** Breeding strategies to generate *Pdk1* cKO/5×FAD mice. *Pdk1*$^{f/f}$ and *Pdk1*$^{f/+}$;*Emx1-Cre* as the control group, *Pdk1*$^{f/f}$;*APP/PS1* and *Pdk1*$^{f/+}$;*Emx1-Cre;APP/PS1* as 5×FAD, *Pdk1*$^{f/f}$;*Emx1-Cre* as *Pdk1* cKO, and *Pdk1*$^{f/f}$;*Emx1-Cre;APP/PS1* as *Pdk1* cKO/5×FAD. **(B)** Representative images of tdTomato/NeuN costaining. Abundant tdTomato+ and tdTomato+/NeuN+ cells were detected in the cortex of *Emx1-Cre;Rosa26-LSL-tdTomato* mice. **(C)** Representative images of fluorescence IHC for PDK1. Sagittal brain sections at 2.5 months were used. The immunoreactivity of PDK1 was qualitatively different between 5×FAD and *Pdk1* cKO/5×FAD mice. The scale bar is 100 μm. **(D)** Western blotting for PDK1. Cortical samples were prepared from 5×FAD and *Pdk1* cKO/5×FAD mice aged at 2.5 months ($n = 3$ mice per group; $^*P < 0.05$).

(Figure 2A and B). Second, quantitative results revealed significant reductions in the average number of plaques in the *Pdk1* cKO/5×FAD cortex at 2.5, 4, and 6 months compared to that in 5×FAD (Figure 2C; $P < 0.001$ at 2.5 months, $P < 0.01$ at 4 months, and $P < 0.001$ at 6 months). Indeed, amyloid plaques were hardly seen in the *Pdk1* cKO/5×FAD cortex (Figure 2A–C). The results suggest that PDK1 deletion may prevent plaque deposition in APP Tg mice.

3.3. Gliosis is alleviated in 5×FAD mice with PDK1 deficiency

Neuroinflammation is a typical feature in mouse models of AD[30,48]. To investigate whether microglia are affected in the *Pdk1* cKO/5×FAD cortex, we performed IHC for Iba1 using brain sections from mice at 2.5, 4, and 6 months. First, Iba1 cortical immunoreactivity did not differ between the control and *Pdk1* cKO mice at each age tested (Figure 3A). However, it was increased in the 5×FAD group at 2.5, 4, and 6 months compared with the control group (Figure 3A and 3B). Second, the average number of Iba1+ cells was significantly higher in the 5×FAD cortex than in the control at 2.5, 4, and 6 months (Figure 3C; $P < 0.001$), but it was significantly lower in the *Pdk1* cKO/5×FAD group at 4 and 6 months compared with the 5×FAD group (Figure 3C; $P < 0.001$). These data suggest that PDK1 deletion may ameliorate microgliosis in APP Tg mice.

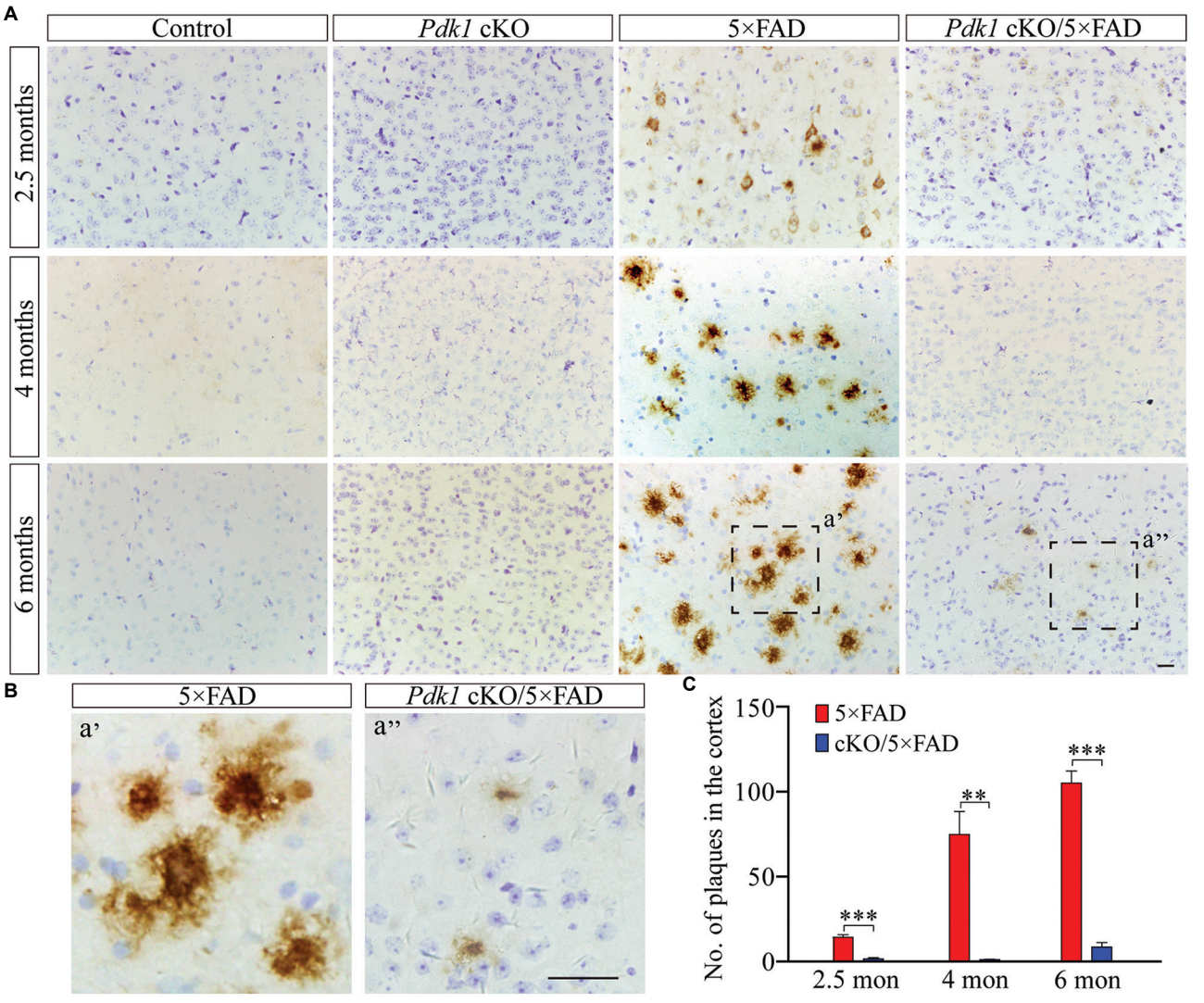


Figure 2. Prevention of Aβ deposition in the cortex of *Pdk1* cKO/5×FAD mice. **(A)** Representative images of Aβ immunostaining. Brain sections at 2.5, 4, and 6 months were used. Images were captured from the cortex of four different groups of mice, including the control, *Pdk1* cKO, 5×FAD, and *Pdk1* cKO/5×FAD groups. The scale bar is 20 μm. **(B)** Enlarged images for boxed regions in **(A)**. The scale bar is 25 μm. **(C)** Quantification results showing the total number of amyloid plaques in the cortex. There were significant differences between 5×FAD and *Pdk1* cKO/5×FAD mice at 2.5 (***$P < 0.001$), 4 (**$P < 0.01$), and 6 months (***$P < 0.001$) (n = 3–5 mice per group per age).

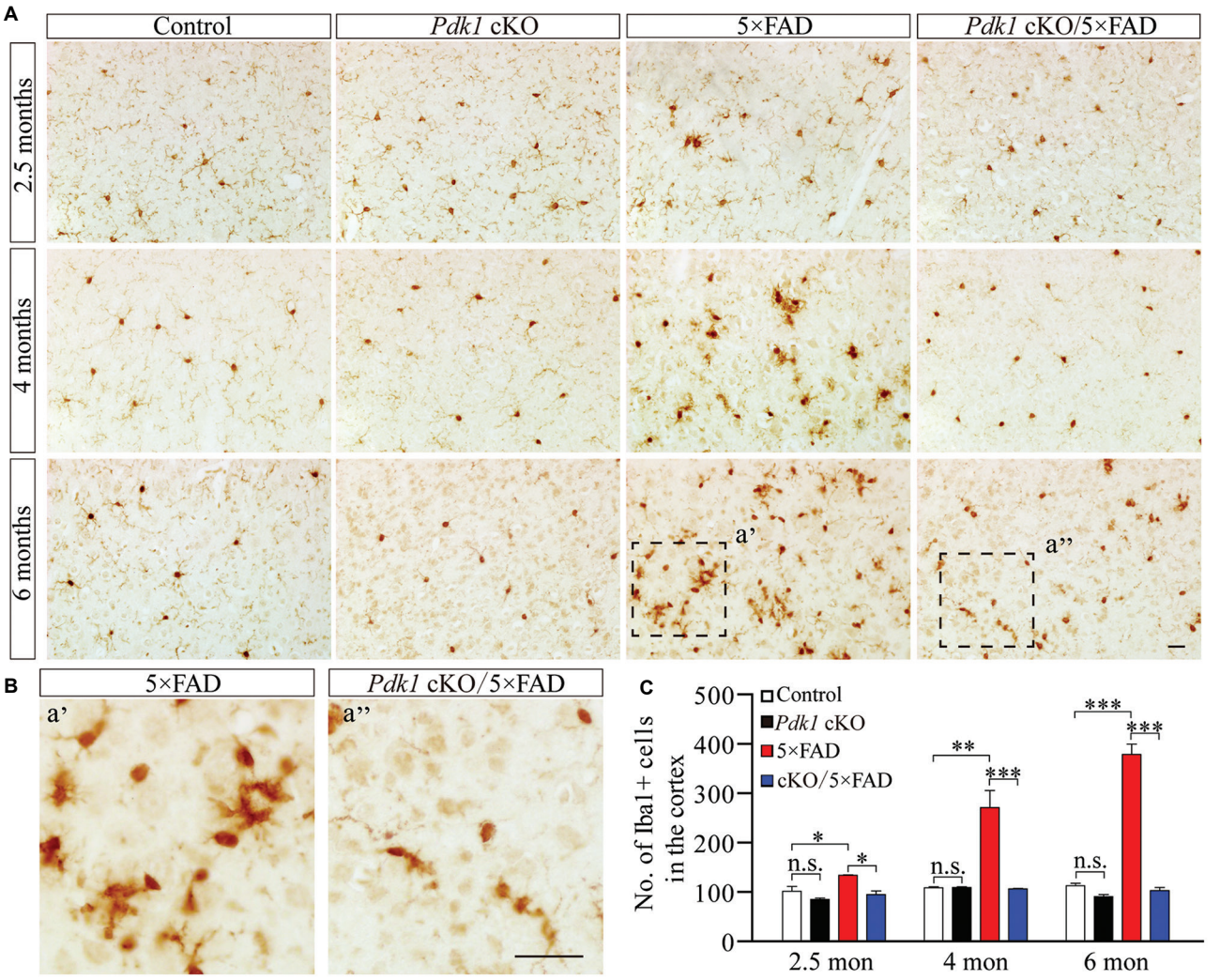


Figure 3. Diminished microgliosis in *Pdk1* cKO/5×FAD mice. **(A)** Representative images of IHC for Iba1 in the cortex. Brain sections were prepared from control, *Pdk1* cKO, 5×FAD, and *Pdk1* cKO/5×FAD mice aged 2.5, 4, and 6 months. The immunoreactivity of Iba1 was qualitatively different between the control and 5×FAD mice. The scale bar is 20 μm. **(B)** Enlarged images for boxed regions in **(A)**. The scale bar is 25 μm. **(C)** Quantification results showing the average number of Iba1+ cells in the cortex. There were significant differences in the number of Iba1+ cells between 5×FAD and *Pdk1* cKO/5×FAD mice at 2.5 (*$P < 0.05$), 4 (***$P < 0.001$), and 6 (***$P < 0.001$) months (n = 3–4 mice per group per age).

To study whether there are changes to astrocytes in *Pdk1* cKO/5×FAD mice, IHC was performed for GFAP. First, it was observed that GFAP immunoreactivity was qualitatively higher in the *Pdk1* cKO cortex and the 5×FAD cortex at 2.5, 4, and 6 months compared with the littermate controls (Figure 4A). GFAP immunoreactivity was reduced in the *Pdk1* cKO/5×FAD cortex compared with the 5×FAD cortex (Figure 4A and B). Second, the total number of GFAP+ cells in the 5×FAD cortex at 4 or 6 months was more than that in the control cortex (Figure 4C; $P < 0.001$), but it was smaller in the *Pdk1* cKO/5×FAD cortex than in the 5×FAD cortex (Figure 4C; $P < 0.001$). These results suggest that astrogliosis may be attenuated by PDK1 deletion in 5×FAD mice.

3.4. mTOR signaling is inhibited in 5×FAD mice with PDK1 deficiency

In the mTOR signaling pathway, S6 kinase (S6K) phosphorylates S6 at Ser235 and Ser236 (Ser235/236) following its activation by mTOR[49,50]. We investigated whether this pathway is affected by PDK1 deletion. First, we performed Western blotting for $pS6^{Ser235/236}$. We found that the levels of $pS6^{Ser235/236}$ were significantly lower in the *Pdk1* cKO/5×FAD cortex than in the 5×FAD cortex (Figure 5A and B). Second, we performed Western blotting for phosphorylated 4E-BP1, an important member of the mTOR pathway[51]. We found that the levels of $p4E\text{-}BP1^{Ser65}$ were lower in the *Pdk1* cKO/5×FAD cortex compared with 5×FAD mice (Figure 5A and B). Third, we performed IHC

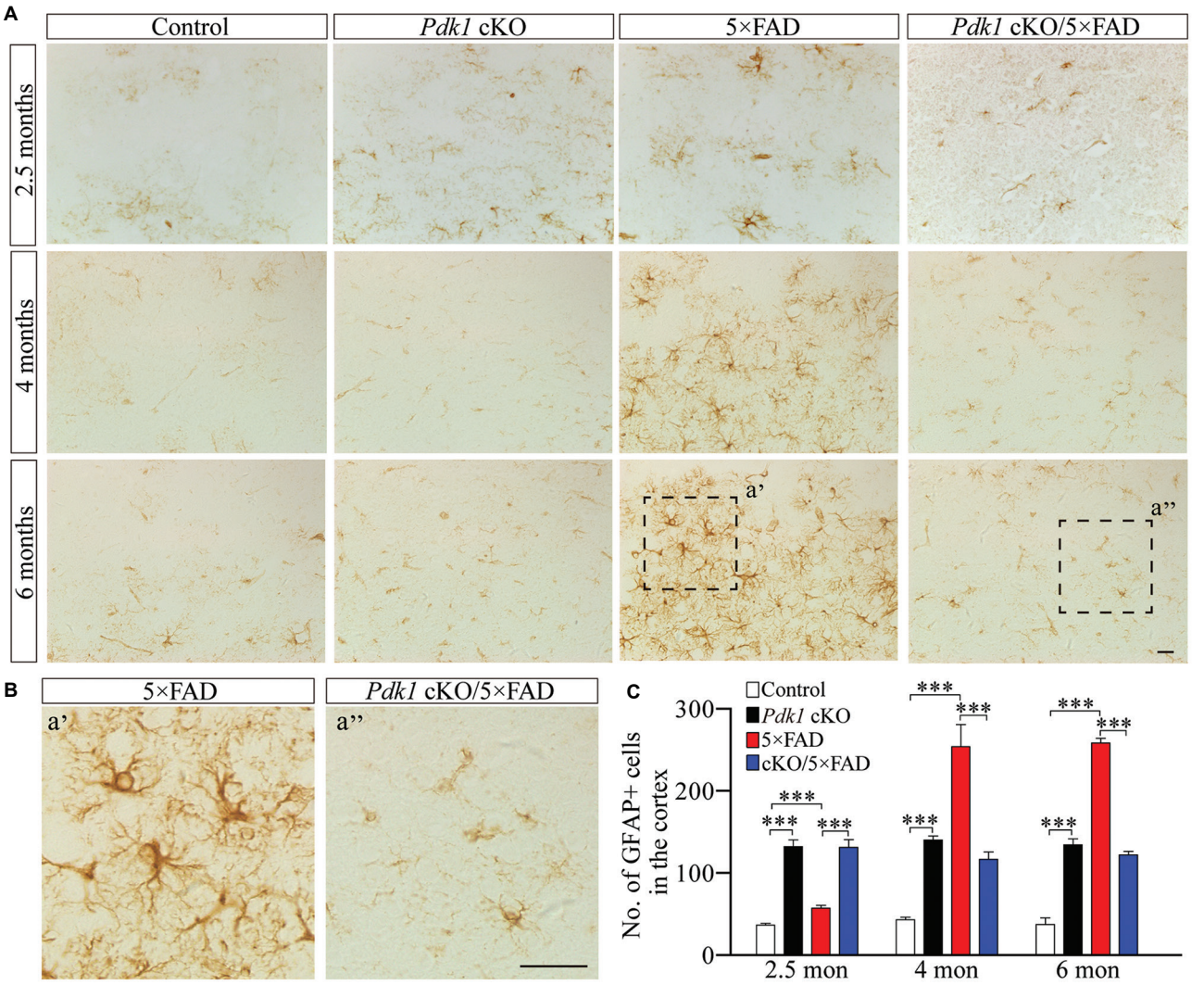


Figure 4. Decreased astrogliosis in *Pdk1* cKO/5×FAD mice. **(A)** Representative images of IHC for GFAP in the cortex. Mice aged at 2.5, 4, and 6 months were used. The immunoreactivity of GFAP was qualitatively increased in 5×FAD mice compared with the controls. The scale bar is 20 μm. **(B)** Enlarged images for boxed regions in **(A)**. The scale bar is 25 μm. **(C)** Quantification results showing the average number of GFAP+ cells in the cortex. There were significant differences in the number of GFAP+ cells between 5×FAD and *Pdk1* cKO/5×FAD mice at 2.5 (***$P < 0.001$), 4 (***$P < 0.001$), and 6 (***$P < 0.001$) months ($n = 3$ mice per group per age).

for pS6$^{Ser235/236}$. Consistent with the biochemical results for pS6, the immunoreactivity of pS6$^{Ser235/236}$ was qualitatively reduced in *Pdk1* cKO/5×FAD mice compared with 5×FAD littermates (Figure 5C). The results are consistent with the notion that PDK1 is an upstream kinase for mTOR[21].

3.5. Expression of APP is decreased in 5×FAD mice with PDK1 deficiency

To determine the molecular mechanisms by which PDK1 deletion reduces Aβ deposition, we focused on APP. Since Aβ is known to be generated from APP through sequential cleavages by β- and γ-secretases[52], Western blotting on APP was performed using cortical samples from four different genotypes of mice (Figure 6A). Total APP levels were robustly higher in the 5×FAD cortex than in the control cortex (Figure 6B) and significantly lower in *Pdk1* cKO/5×FAD mice than in 5×FAD mice (Figure 6B). Next, IHC for APP was performed. The results showed that APP immunoreactivity was qualitatively reduced in *Pdk1* cKO/5×FAD mice than in 5×FAD mice (Figure 6C).

We also performed Western blotting to examine the products of APP cleaved by α- and γ-secretases: Secreted (s)APPα and APP-C-terminal fragment (CTF) (Figure 6D). Our results revealed that protein levels for sAPPα and APP-CTF were significantly lower in *Pdk1* cKO/5×FAD mice than in 5×FAD mice

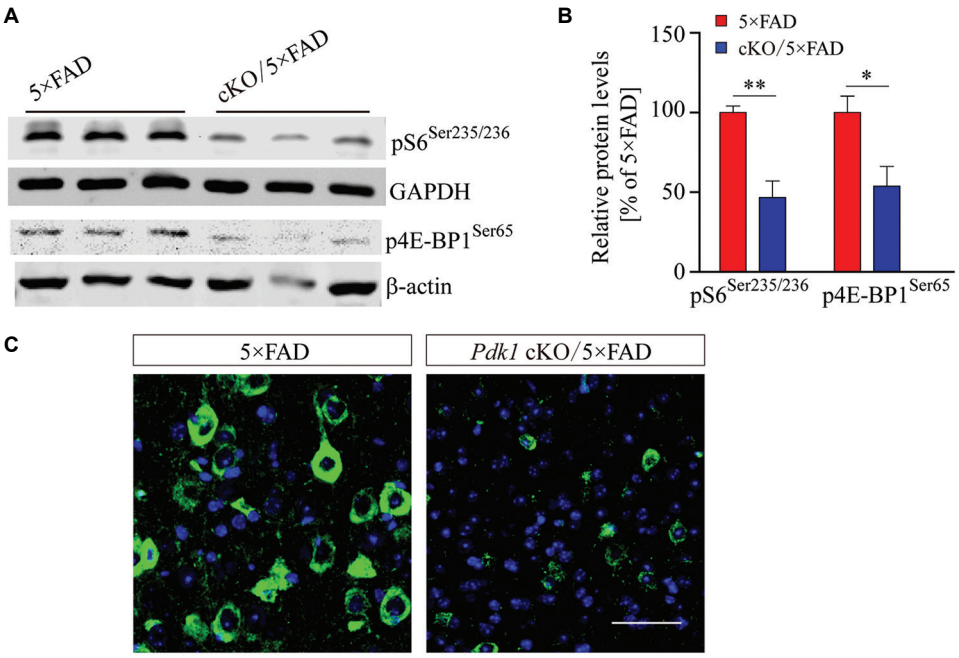


Figure 5. Reduced mTOR activity in *Pdk1* cKO/5×FAD mice. **(A and B)** Western blotting for $pS6^{Ser235/236}$ and $p4E\text{-}BP1^{Ser65}$. **(A)** Cortical samples from 5×FAD and *Pdk1* cKO/5×FAD mice were used. **(B)** There were significant differences in the relative levels of $pS6^{Ser235/236}$ and $p4E\text{-}BP1^{Ser65}$ between 5×FAD and *Pdk1* cKO/5×FAD mice at 4 months (n = 3–4 mice per group; $^{*}P < 0.05$, $^{**}P < 0.01$). **(C)** Fluorescence IHC for $pS6^{Ser235/236}$. Brain sections at 4 months were used. The immunoreactivity of $pS6^{Ser235/236}$ was qualitatively different between 5×FAD and *Pdk1* cKO/5×FAD mice. The scale bar is 50 μm.

(Figure 6D and 6E). These findings are consistent with those for total APP (Figure 6A). Moreover, we found that the ratio of sAPPα to total APP was similar between 5×FAD and *Pdk1* cKO/5×FAD mice (Figure 6F). Since α- and β-secretases are responsible for Aβ generation[53], we performed Western blotting for a disintegrin and metalloproteinase 10 (ADAM10) and beta-site APP cleaving enzyme 1 (BACE1). However, the levels of these two proteins did not significantly differ in 5×FAD and *Pdk1* cKO/5×FAD mice (Figure 6G and H).

4. Discussion

The recent study by Petri *et al.* suggests that pharmacological inhibition on PDK1 may be beneficial to AD. The aim of this study was to test whether early inhibition of PDK1 may prevent AD-like pathology in APP Tg mice. In this study, *Emx1-Cre*-mediated *Pdk1* cKO/5×FAD mice were used. We demonstrated that PDK1 deletion prevents plaque deposition and attenuates gliosis in the 5×FAD cortex. In addition, PDK1 deletion inhibits the mTOR signaling pathway and downregulates APP expression in 5×FAD mice. Overall, these findings are consistent with the notion that PDK1 could be a promising therapeutic target for AD.

As previously reported, long-term treatment with BX912 results in the death of Tg2576 mice[22]. In line with this, straight KO of *Pdk1* leads to early embryonic lethality in mice[27]. In contrast, *Emx1-Cre*-mediated PDK1 deletion does not cause any lethal effect in mice. We did not observe any abnormal deaths in *Pdk1* cKO/5×FAD mice or in *Pdk1* cKO mice. Nevertheless, the pharmacological method[22] and genetic approach used in the present study may efficiently inhibit PDK1 function. However, it is known that *Emx1-Cre*-mediated PDK1 deletion disrupts the formation of cortex in mice[28]. Therefore, we cannot exclude the possibility that a reduced cortex size may play a role in improving AD-like pathology in *Pdk1* cKO/5×FAD mice.

Genetic approaches have been widely used in neurodegenerative diseases[54]. First, a gene therapy-based clinical trial has revealed high treatment efficacy in spinal muscular atrophy (SMA) type 1[55]. Second, genetic approaches have been tested in animal models for amyotrophic lateral sclerosis[56] and Huntington's disease[57]. Third, the CRISPR-Cas9 technology was employed in a study to inactivate the mutant human *APP* gene in 5×FAD mice, resulting in a significant alleviation of AD-like pathology[58]. Based on our finding that early inhibition of PDK1 prevents AD-like pathology in 5×FAD mice, we propose the development of a genetic approach that targets neuronal PDK1 as a potential treatment strategy for AD.

To identify the underlying molecular mechanisms in *Pdk1* cKO/5×FAD mice, we examined the expression of APP and APP processing enzymes, including α-secretase

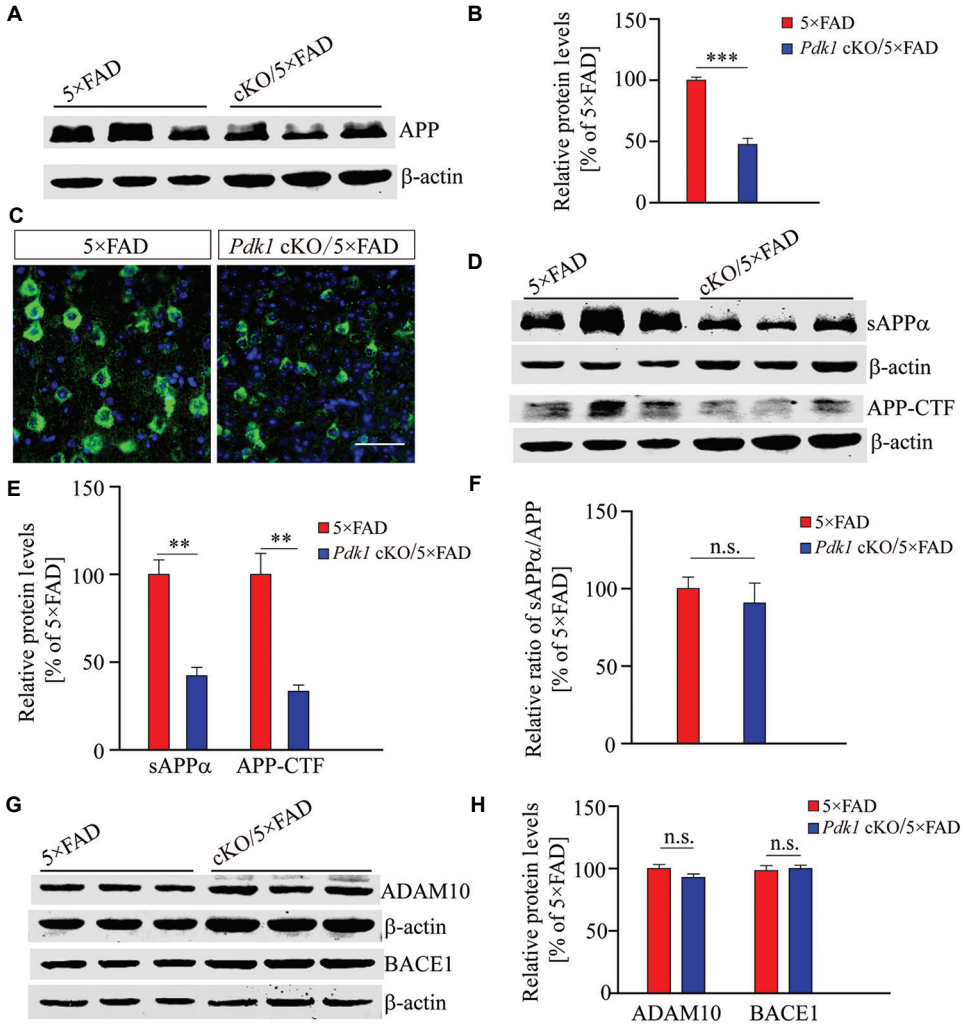


Figure 6. Reduced amyloid precursor protein (APP) expression in *Pdk1* cKO/5×FAD mice. **(A)** Western blotting for full-length APP (APP-FL). Cortical samples from 5×FAD and *Pdk1* cKO/5×FAD mice were used. β-actin was used as the loading control. **(B)** Quantification results of APP-FL. There was a significant difference in the APP levels between 5×FAD and *Pdk1* cKO/5×FAD mice ($n = 3$ mice per group; $^{***}P < 0.001$). **(C)** Fluorescence IHC for APP. Brain sections at 4 months were used. The immunoreactivity of APP was qualitatively reduced in *Pdk1* cKO/5×FAD mice compared with 5×FAD mice. The scale bar is 50 μm. **(D)** Western blotting for sAPPα and APP-CTF. Cortical samples from 5×FAD and *Pdk1* cKO/5×FAD mice at 4 months were used. β-actin served as the loading control. **(E)** Quantification results of sAPPα and APP-CTF. There were significant differences in the levels of sAPPα and APP-CTF between 5×FAD and *Pdk1* cKO/5×FAD mice ($^{**}P < 0.01$; $n = 3$ mice per group). **(F)** Quantification results showing the ratio of sAPPα to APP. There was no significant difference between 5×FAD and *Pdk1* cKO/5×FAD mice (n.s., not significant; $n = 3$ mice per group). **(G)** Western blotting for ADAM10 and BACE1. β-actin was used as the loading control. **(H)** Quantification results of ADAM10 and BACE1. There were no significant differences in the levels of ADAM10 and BACE1 between 5×FAD and *Pdk1* cKO/5×FAD mice (n.s., not significant; $n = 3$ mice per group).

and BACE1. Since the levels of APP were significantly reduced in the *Pdk1* cKO/5×FAD cortex, we reason that this may be responsible for the prevention of plaque deposition. In agreement with this, Duan *et al.* have reported that CRISPR-Cas9-mediated KO of mutant human *APP* gene reduces amyloid plaques and improves spatial working memory in 5×FAD mice.

On the other hand, no changes have been observed in the levels of ADAM10, BACE1, and sAPPα in *Pdk1* cKO/5×FAD cortices, suggesting a possibility that PDK1 deletion may not enhance the activity of α-secretase or β-secretase. In contrast, evidence has shown that BX912 may promote α-secretase-dependent cleavage of APP[22]. We reason that the above discrepancy reflects the fact that pharmacological and genetic approaches exhibit different target selectivity and cell specificity[37]. For instance, BX912 is known to inhibit a spectrum of kinases, including PDK1[59,60]. In contrast, *Emx1-Cre*-mediated gene recombination permits conditional inactivation of PDK1 in neurons in the postnatal forebrain.

Since ribosomal kinase S6 is a key member of the mTOR signaling pathway[15,16,21], the decreased pS6 levels suggest that the activity of mTOR may be reduced in the *Pdk1* cKO/5×FAD cortex. Since previous evidence has shown that S6 is essential for ribosome biogenesis[61], it is likely that ribosome biogenesis may be regulated by PDK1 via S6[37]. In essence, the following molecular and cellular mechanisms may be responsible for the prevention effects on AD-like pathology as observed in *Pdk1* cKO/5×FAD mice. First, PDK1 deletion causes a reduction in mTOR signaling and subsequently inhibits the activity of S6. Second, the deactivation of S6 may impair ribosome-dependent protein synthesis, which then downregulates APP expression at the translational level[37]. Third, Aβ generation from APP is repressed, and gliosis is ameliorated in the forebrain of *Pdk1* cKO/5×FAD mice.

5. Conclusion

Deletion of PDK1 in the developing cortex prevents plaque deposition and ameliorates gliosis in 5×FAD mice. PDK1 deletion inhibits mTOR signaling activity and downregulates APP expression. PDK1 may serve as a potential target for the treatment of AD.

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Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

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Formal analysis: Xiaolian Ye, Liyang Yao, Wenhao Chen, and Wenkai Shao

Investigation: Xiaolian Ye, Liyang Yao, Wenhao Chen, and Wenkai Shao

Methodology: Xiaolian Ye, Liyang Yao, Wenhao Chen, and Wenkai Shao

Writing – original draft: Xiaolian Ye and Guiquan Chen

Writing – review & editing: Guiquan Chen

Ethics approval and consent to participate

An animal protocol (AP) for this study was approved by the Institutional Animal Care and Use Committee (IACUC) of the MARC at Nanjing University.

Consent for publication

Not applicable.

Availability of data

The experimental materials used in this study are available from corresponding authors on reasonable request.

References

1. Li T, Han Y, 2022, Insights on amyloid-related imaging abnormalities from the "Pre-Alzheimer's Disease Alliance of China". *Adv Neurol*, 1: 2.
2. Scheltens P, Blennow K, Breteler MM, *et al.*, 2016, Alzheimer's disease. *Lancet*, 388: 505–517.
3. Horsburgh K, Holland P, Reimer M, *et al.*, 2011, Axon-glial disruption: The link between vascular disease and Alzheimer's disease? *Biochem Soc Trans*, 39: 881–885.
4. Frisoni GB, Altomare D, Thal DR, *et al.*, 2022, The probabilistic model of Alzheimer disease: The amyloid hypothesis revised. *Nat Rev Neurosci*, 23: 53–66.
5. Hardy, J, Selkoe DJ, 2002, The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science*, 297: 353–356.
6. Goedert M, Eisenberg DS, Crowther RA, 2017, Propagation of Tau aggregates and neurodegeneration. *Ann Rev Neurosci*, 40: 189–210.
7. Mintun MA, Lo AC, Evans CD, *et al.*, 2021, Donanemab in early Alzheimer's disease. *N Engl J Med*, 384: 1691–1704.
8. Sevigny J, Chiao P, Bussiere T, *et al.*, 2016, The antibody aducanumab reduces A beta plaques in Alzheimer's disease. *Nature*, 537: 50–56.
9. Peng Y, Hu Y, Xu S, *et al.*, 2012, L-3-n-butylphthalide reduces tau phosphorylation and improves cognitive deficits in AbetaPP/PS1-Alzheimer's transgenic mice. *J Alzheimers Dis*, 29: 379–391.
10. Peng Y, Sun J, Hon S, *et al.*, 2010, L-3-n-butylphthalide improves cognitive impairment and reduces amyloid-beta in a transgenic model of Alzheimer's disease. *J Neurosci*, 30: 8180–8189.
11. Chen G, Chen KS, Kobayashi D, *et al.*, 2007, Active-amyloid immunization restores spatial learning in PDAPP mice displaying very low levels of beta-amyloid. *J Neurosci*, 27: 2654–2662.
12. Schenk D, Barbour R, Dunn W, *et al.*, 1999, Immunization with amyloid-attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*, 400: 173–177.

13. Yao YL, Wang YX, Yang FC, *et al.*, 2022, Targeting AKT and CK2 represents a novel therapeutic strategy for SMO constitutive activation-driven medulloblastoma. *CNS Neurosci Ther*, 28: 1033–1044.

14. Wang J, Yu Z, Tao Y, *et al.*, 2021, A novel palmitic acid hydroxy stearic acid (5-PAHSA) plays a neuroprotective role by inhibiting phosphorylation of the m-TOR-ULK1 pathway and regulating autophagy. *CNS Neurosci Ther*, 27: 484–496.

15. Wang H, Liu M, Zou G, *et al.*, 2021, Deletion of PDK1 in oligodendrocyte lineage cells causes white matter abnormality and myelination defect in the central nervous system. *Neurobiol Dis*, 148: 105212.

16. Wang H, Liu M, Ye Z, *et al.*, 2021, Akt regulates Sox10 expression to control oligodendrocyte differentiation via phosphorylating FoxO1. *J Neurosci*, 41: 8163–8180.

17. Ma Y, Xu X, Li C, *et al.*, 2021, Induced neural progenitor cell-derived extracellular vesicles promote neural progenitor cell survival via extracellular signal-regulated kinase pathway. *CNS Neurosci Ther*, 27: 1605–1609.

18. Fedder-Semmes KN, Appel B, 2021, The Akt-mTOR pathway drives myelin sheath growth by regulating Cap-dependent translation. *J Neurosci*, 41: 8532.

19. Bonet IJ, Khomula EV, Araldi D, *et al.*, 2021, PI3K/AKT signaling in high molecular weight hyaluronan (HMWH)-induced anti-hyperalgesia and reversal of nociceptor sensitization. *J Neurosci*, 41: 8414.

20. Zhao XF, Liao Y, Alam MM, *et al.*, 2020, Microglial mTOR is neuronal protective and antiepileptogenic in the pilocarpine model of temporal lobe epilepsy. *J Neurosci*, 40: 7593.

21. Fruman DA, Chiu H, Hopkins BD, *et al.*, 2017, The PI3K pathway in human disease. *Cell*, 170: 605–635.

22. Pietri M, Dakowski C, Hannaoui S, *et al.*, 2013, PDK1 decreases TACE-mediated alpha-secretase activity and promotes disease progression in prion and Alzheimer's diseases. *Nat Med*, 19: 1124–1131.

23. Pei JJ, Khatoon S, An WL, *et al.*, 2003, Role of protein kinase B in Alzheimer's neurofibrillary pathology. *Acta Neuropathol*, 105: 381–392.

24. Mathys H, Davila-Velderrain J, Peng Z, *et al.*, 2019, Single-cell transcriptomic analysis of Alzheimer's disease. *Nature*, 570: 332–337.

25. Yang S, Pascual-Guiral S, Ponce R, *et al.*, 2018, Reducing the levels of Akt activation by PDK1 knock-in mutation protects neuronal cultures against synthetic smyloid-beta peptides. *Front Aging Neurosci*, 9, 435.

26. Stein TD, Johnson, JA, 2002, Lack of neurodegeneration in transgenic mice overexpressing mutant amyloid precursor protein is associated with increased levels of transthyretin and the activation of cell survival pathways. *J Neurosci*, 22: 7380.

27. Lawlor MA, Mora A, Ashby PR, *et al.*, 2002, Essential role of PDK1 in regulating cell size and development in mice. *EMBO J*, 21: 3728–3738.

28. Xu C, Yu L, Hou J, *et al.*, 2017, Conditional deletion of PDK1 in the forebrain causes neuron loss and increased apoptosis during cortical development. *Front Cell Neurosci*, 11: 330.

29. Wei YJ, Han XN, Zhao CJ, 2020, PDK1 regulates the survival of the developing cortical interneurons. *Mol Brain*, 13: 65.

30. Oakley H, Cole SL, Logan S, *et al.*, 2006, Intraneuronal-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. *J Neurosci*, 26: 10129–10140.

31. Liu T, Zhu X, Huang C, *et al.*, 2022, ERK inhibition reduces neuronal death and ameliorates inflammatory responses in forebrain-specific *Ppp2cα* knockout mice. *FASEB J*, e222515.

32. Huang C, Liu T, Wang Q, *et al.*, 2020, Loss of PP2A disrupts the retention of radial glial progenitors in the telencephalic niche to impair the generation for late-born neurons during cortical development. *Cereb Cortex*, 30: 4183–4196.

33. Cheng S, Liu T, Hu, Y, *et al.*, 2019, Conditional inactivation of Pen-2 in the developing neocortex leads to rapid switch of apical progenitors to basal progenitors. *J Neurosci*, 39: 2195–2207.

34. Englund C, Fink A, Lau C, *et al.*, 2005, Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *J Neurosci*, 25: 247–251.

35. Wu J, Shao C, Ye X, *et al.*, 2021, *In vivo* brain imaging of amyloid-aggregates in Alzheimer's disease with a near-infrared fluorescent probe. *ACS Sensors*, 6: 863–870.

36. Ma X, Wang Y, Hua J, *et al.*, 2020, Abeta-sheet-targeted theranostic agent for diagnosing and preventing aggregation of pathogenic peptides in Alzheimer's disease. *Sci CHINA Chem*, 63: 73–82.

37. Ye X, Chen L, Wang H, *et al.*, 2022, Genetic inhibition of PDK1 robustly reduces plaque deposition and ameliorates gliosis in the 5×FAD mouse model of Alzheimer's disease. *Neuropathol Appl Neurobiol*, 48: e12839.

38. Han X, Wei Y, Ba R, *et al.*, 2021, PDK1 regulates the lengthening of G1 phase to balance RGC proliferation and differentiation during cortical neurogenesis. *Cereb Cortex*, 32: 3488–3500.

39. Han X, Wei Y, Wu X, *et al.*, 2020, PDK1 regulates transition period of apical progenitors to basal progenitors by controlling asymmetric cell division. *Cereb Cortex*, 30: 406–420.

40. Wang L, Cheng S, Yin Z, *et al.*, 2015, Conditional inactivation of Akt three isoforms causes tau hyperphosphorylation in the brain. *Mol Neurodegener*, 10: 33.

41. Li QQ, Chen J, Hu P, *et al.*, 2022, Enhancing GluN2A-type NMDA receptors impairs long-term synaptic plasticity and

learning and memory. *Mol Psychiatry*: s41380-022-01579-7.

42. Peng SX, Wang YY, Zhang M, *et al.*, 2021, SNP rs10420324 in the AMPA receptor auxiliary subunit TARP γ-8 regulates the susceptibility to antisocial personality disorder. *Sci Rep*, 11: 11997.

43. Xia Y, Zhang Y, Xu M, *et al.*, 2022, Presenilin enhancer2 is crucial for the transition of apical progenitors into neurons but into not basal progenitors in the developing hippocampus. *Development*, 149: dev200272.

44. Teng X, Hu P, Chen Y, *et al.*, 2022, A novel Lgi1 mutation causes white matter abnormalities and impairs motor coordination in mice. *FASEB J*, 36: e22212.

45. Hou J, Bi H, Ye Z, *et al.*, 2021, Pen-2 negatively regulates the differentiation of oligodendrocyte precursor cells into astrocytes in the central nervous system. *J Neurosci*, 41: 4976–4990.

46. Bi H, Zhou C, Zhang Y, *et al.*, 2021, Neuron-specific deletion of presenilin enhancer2 causes progressive astrogliosis and age-related neurodegeneration in the cortex independent of the Notch signaling. *CNS Neurosci Ther*, 27: 174–185.

47. Hou J, Bi H, Ge Q, *et al.*, 2022, Heterogeneity analysis of astrocytes following spinal cord injury at single-cell resolution. *FASEB J*, 36: e22442.

48. Saura CA, Chen G, Malkani S, *et al.*, 2005, Conditional inactivation of presenilin 1 prevents amyloid accumulation and temporarily rescues contextual and spatial working memory impairments in amyloid precursor protein transgenic mice. *J Neurosci*, 25: 6755–6764.

49. Lai KO, Liang ZY, Fei EK, *et al.*, 2015, Cyclin-dependent kinase 5 (Cdk5)-dependent phosphorylation of p70 ribosomal S6 kinase 1 (S6K) is required for dendritic spine morphogenesis. *J Biol Chem*, 290: 14637–14646.

50. Ruvinsky I, Meyuhas O, 2006, Ribosomal protein S6 phosphorylation: From protein synthesis to cell size. *Trends Biochem Sci*, 31: 342–348.

51. Hay N, Sonenberg N, 2004, Upstream and downstream of mTOR. *Genes Dev*, 18: 1926–1945.

52. Kimberly WT, LaVoie MJ, Ostaszewski BL, *et al.*, 2003, Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. *Proc Natl Acad Sci USA*, 100: 6382–6387.

53. De Strooper B, Karran E, 2016, The cellular phase of Alzheimer's disease. *Cell*, 164: 603–615.

54. Anguela XM, High KA, 2019, Entering the modern era of gene therapy. *Ann Rev Med*, 70: 273–288.

55. Mendell JR, Al-Zaidy S, Shell R, *et al.*, 2017, Single-dose gene-replacement therapy for spinal muscular atrophy. *N Engl J Med*, 377: 1713–1722.

56. Thomsen GM, Gowing G, Latter J, *et al.*, 2014, Delayed disease onset and extended survival in the SOD1G93A rat model of amyotrophic lateral sclerosis after suppression of mutant SOD1 in the motor cortex. *J Neurosci*, 34: 15587.

57. McBride JL, Pitzer MR, Boudreau RL, *et al.*, 2011, Preclinical safety of RNAi-mediated HTT suppression in the rhesus macaque as a potential therapy for Huntington's disease. *Mol Ther*, 19: 2152–2162.

58. Duan YY, Ye T, Qu Z, *et al.*, 2022, Brain-wide Cas9-mediated cleavage of a gene causing familial Alzheimer's disease alleviates amyloid-related pathologies in mice. *Nat Biomed Eng*, 6: 168–180.

59. Peifer C, Alessi DR, 2008, Small molecule inhibitors of PDK1. *ChemMedChem*, 3: 1810–1838.

60. Bain J, Plater L, Elliott M, *et al.*, 2007, The selectivity of protein kinase inhibitors: A further update. *Biochem J*, 408: 297–315.

61. Pelletier J, Thomas G, Volarević S, 2018, Ribosome biogenesis in cancer: New players and therapeutic avenues. *Nat Rev Cancer*, 18: 51–63.

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REVIEW ARTICLE

Gut-brain axis in depression: Crosstalk between neuroinflammation and gut microbiota

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Abstract

Depression is the most common mental disorder and the leading cause of disability and suicide worldwide. Recently, during the COVID-19 pandemic, there has been a dramatic increase in the number of patients with depression on a global scale. Therefore, new insights into the underlying pathophysiology of depression are urgently required to develop more effective therapeutic strategies. An interesting fact is the coexistence of increased intestinal permeability and disrupted blood-brain barrier observed in patients with depression and animal models. A growing number of studies have revealed that a bidirectional interaction exists between the brain and the gastrointestinal tract, commonly termed the gut-brain axis. Emerging evidence has suggested that the regulation of neuroinflammation and gut homeostasis through the gut-brain axis is influenced by stress and depression, in which gut microbiota and microbe-derived short-chain fatty acids play a crucial role. Here, we describe the molecular and cellular mechanisms involved in the crosstalk between neuroinflammation and gut microbiota in depression. We further highlight fecal microbiota transplantation and dietary supplementation in depression and discuss their potential as therapeutic targets for depression.

Keywords: Depression; Stress; Gut-brain axis; Microbiota; Short-chain fatty acids; Neuroinflammation; Nutrients

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1. Introduction

Mental health disorders affect around 15% of the population during their lifetime, with depression being one of the most common[1]. As a result of the COVID-19 pandemic, the number of patients with major depressive disorder (MDD) increased by 28% in 2020[2]. Globally, it is estimated that 5% of adults and 5.7% of elderly over the age of 60 suffer from depression. Patients with depression may suffer immensely, and in severe conditions, depression may even lead to suicide[3]. The antidepressants that are currently in use include older drug types, such as monoamine oxidase inhibitors and tricyclic antidepressants, as well as newer drug types, such as selective serotonin

reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors. Non-pharmacological therapy, such as cognitive therapy, is also used when medications fail to meet the treatment outcome[4]. Unfortunately, our knowledge of depression and our ability to treat it effectively is lacking; for example, despite temporary remission with antidepressant medication, patients either have a high risk of relapse or do not adequately respond to pharmacological treatment[4]. Therefore, new insights into the underlying pathophysiology of depression are urgently required to develop more effective therapeutic strategies.

Neuroinflammation is known to be associated with a variety of neurological diseases, such as Alzheimer's disease[5] and Parkinson's disease[6]. Inflammatory processes are implicated in the pathophysiology of depression. Numerous studies have suggested a strong association between depression and pro-inflammatory mediators in peripheral blood and cerebrospinal fluid (CSF)[7] as well as an association between the therapeutic application of interferon (IFN)-α and depression in 30 – 50% of patients[8,9]. According to a meta-analysis, patients with MDD have higher levels of interleukin (IL)-6 and C-reactive protein (CRP) compared to non-depressed subjects[10]. Another meta-analysis based on clinical trials has shown that pro-inflammatory cytokine inhibitors (adalimumab, etanercept, infliximab, and tocilizumab) can alleviate depressive symptoms in patients[11]. In addition, the leakage of the blood-brain barrier (BBB) observed in depression is partly due to the persistent low-grade inflammation present in depressive states. Inflammation is likely a critical disease modifier that promotes susceptibility to depression. However, the exact mechanism of the interaction between inflammation and depression remains unclear[7].

The gut-brain axis is a potential area of research as there is mounting evidence showing that the gut microbiota has a strong influence on emotional behavior and neurological processes[12]. The human gut is home to nearly 100 trillion bacteria that are essential for maintaining health[13]. In the last decades, a growing number of evidence has suggested that the gut-brain axis is involved in the pathophysiology of neurodevelopmental and neurological disorders, where the gut microbiota is a key regulator in the gut-brain axis[14,15]. Alterations in gut microbiota composition and subsequent metabolites of short-chain fatty acids (SCFAs) have been increasingly observed in patients with depression[16]. Similarly, an imbalance of gut microbiota has also been discovered in animal models with depression-like behavior[17]. Interestingly, probiotic formulations have shown anxiolytic-like activity in a rat model and led to the remission of psychological distress in volunteers of a clinical trial[18].

Overall, depression is characterized by low-grade systemic and neurological inflammation, leakage of the BBB and intestinal mucosal barrier, a high relative abundance of the pro-inflammatory species of gut microbiota, and a low abundance of SCFA-producing probiotics (Figure 1). To meet the current requirements for depression/MDD-targeted treatment strategies, the present review provides a necessary update to the expanding literature describing the characterization and interaction between gut microbiota and neuroinflammation in depression. This review also aims to integrate information and explore the potential mechanisms for modulating gut microbiota homeostasis in the treatment of depression.

2. Changes in gut homeostasis as a component of depression

2.1. Leaky gut and inflammation

The intestinal barrier is a dynamic dense entity that provides a strong physical barrier. It interacts with and responds to various stimuli, such as inhibiting pathogen colonization through the production of antimicrobial substances and preventing bacterial adhesion through the secretion of immunoglobulin A (Ig A) as well as glycocalyx and mucus[19]. However, an increased intestinal permeability may underlie the persistent low-grade inflammation observed in neurological disorders, such as depression[20]. In a study, there was increased intestinal fatty acid binding protein (FABP), a marker of enterocyte damage, in the blood of recent suicidal patients. The high level of intestinal FABP was found to be related to the severity of depressive symptoms and suicidal symptoms. Moreover, the level of IL-6 was also increased in the blood of recent suicidal patients and found to be correlated with two gut permeability markers: zonulin and FABP[20]. Psychological stress has been shown to increase small intestinal permeability in humans[21]. Camilleri and Clark *et al.* have pointed out that there is a strong correlation between stress and changes in gut microbiota composition, including a decrease in the composition of commensal bacteria and an increase in the level of inflammation, disrupting the integrity and permeability of the intestine and causing leaky gut[19,22]. Specifically, the increased translocation of bacterial toxins and food particles due to a compromised gut barrier has been associated with the activation of the immune system and the production of inflammatory mediators[19]. A leaky gut allows the translocation of lipopolysaccharide (LPS) from the gut into the circulation, activating immune cells and leading to an increased release of pro-inflammatory mediators and systemic hypo-inflammation[23]. It has been shown that stress increased paracellular permeability in the colon,

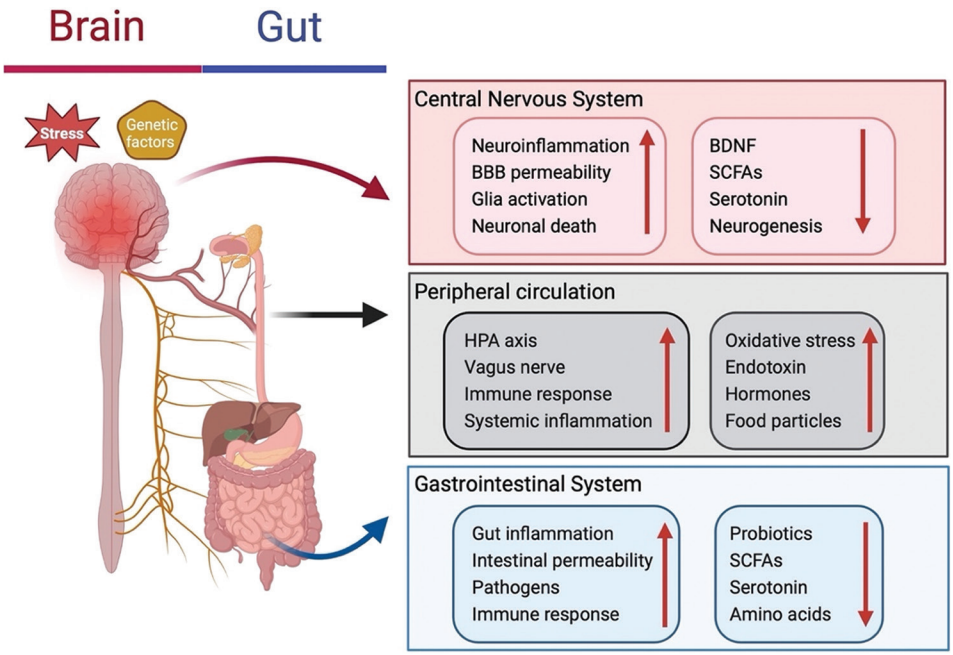


Figure 1. Role and mechanisms of the gut-brain axis in depression. Depression is characterized by low-grade systemic and neurological inflammation, lack of serotonin and brain-derived neurotrophic factor (BDNF), leakage of the BBB and intestinal mucosal barrier, a high relative abundance of the pro-inflammatory species of gut microbiota, and a low abundance of SCFA-producing probiotics. Increased and sustained stress, infections, genetics, or other co-factors may lead to dysregulation of gut homeostasis, including leaky gut, immune and inflammatory hyper responsiveness, and dysbiosis of the gut microbiota and metabolism. Depression is bidirectionally regulated by the gut-brain axis via the HPA axis, vagus nerve, inflammatory signals, immune signals, and chemical signals.
BBB: Blood-brain barrier; BDNF: Brain-derived neurotrophic factor; HPA: Hypothalamic-pituitary-adrenal; SCFAs: Short-chain fatty acids. Created with BioRender.com.

endotoxemia in blood, and pro-inflammatory cytokines (IL-1β, IL-6, and tumor necrosis factor [TNF]-α) in the paraventricular nucleus of the hypothalamus in a rat model[24]. In a clinical study with 50 MDD patients and 30 healthy subjects, bacterial translocation was observed in MDD patients, as indicated by an elevated expression of bacterial DNA in the blood. Furthermore, the activation of toll-like receptor 4/nuclear factor-κB (TLR-4/NF-κB) pro-inflammatory pathway and the increased levels of IL-6 and CRP were observed in MDD patients[25].

2.2. Dysbiosis of gut microbiota

The gut is enriched with several bacterial phyla, of which the two most abundant phyla are *Firmicutes* and *Bacteroides*, accounting for more than 70% of the gut microbiome[26]. In contrast, *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* are fewer in numbers[26]. Simpson *et al.* have summarized the data from 19 case–control studies and showed that the abundance of *Actinobacteria* was higher in MDD patients in six studies and was positively associated with depressive symptoms; a lower abundance of *Bacteroides* was observed in MDD patients in seven studies, while the other two studies showed the opposite[12]. Jiang *et al.* have analyzed fecal samples from 46 patients with depression and 30 healthy subjects and found that the abundance of *Bacteroides*, *Proteobacteria*, and *Actinobacteria* increased in MDD patients compared to healthy controls, while the abundance of *Firmicutes* decreased[16]. Based on the different gut microbial compositions in mood disorders, the gut microbiome was used to distinguish depressive episodes of bipolar disorder from those of MDD in a large clinical study with 165 subjects by 16S rRNA gene sequence analysis. MDD was characterized by alterations in *Firmicutes* and *Bacteroides* phyla, *Bacteroidaceae* and *Lachnospiraceae* family, and *Bacteroides, Ruminococcus_torques*, and *Ruminococcus_gnavus* genus, whereas bipolar disorder was mainly associated with alterations in *Bacteroides*, *Roseburia*, *Coprococcus*, *Prevotella_9*, and *Pseudomonas* genus[27,28]. The same researchers performed a cross-sectional whole-genome shotgun metagenomics analysis of fecal samples from 156 patients with MDD and 155 healthy controls and found that the *Bacteroides* genus was mainly enriched but *Blautia* and *Eubacterium* genus were mainly depleted in patients with MDD[29]. It has been reported that the upregulation of *Bacteroides* contributes to higher peripheral cytokine concentrations and increased

systemic inflammation in patients with Parkinson's disease[30], while *Blautia* species may be associated with good cognition and decreased inflammation in hepatic encephalopathy[31]. In a study, the transplantation of gut microbiota from patients with rheumatoid arthritis activated an abnormal differentiation of T cells in germ-free (GF) mice, thereby inducing depression-like behavior[32]. Moreover, two animal studies have shown that fecal microbiota transplantation from MDD patients to GF mice or microbiota-depleted rats can induce depression-like behaviors, thus elucidating the role of the pro-inflammatory species of gut microbiota in the pathology of depression[28,33]. Consistently, the transplantation of fecal microbiota from NOD-like receptor protein 3 (NLRP3) knock-out (KO) mice reduced the depression-like behavior induced by chronic unpredictable stress in recipient mice in our previous study, which was found to be related to the changes in the relative abundance of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* phyla[34]. However, due to large individual variability, methodological heterogeneity, and dietary diversity, we are only beginning to fully understand the implications of the gut microbiome on human mental health and neuropsychiatric disorders[35].

3. Inflammatory pathways across the gut-brain axis

3.1. Systemic circulation pathway

Inflammation has been widely discussed in reviews of brain diseases involving the gut-brain axis, such as the interaction of central nervous system (CNS)-resident and peripheral immune pathways[36], the relationship between inflammasome and gut microbiota[37], as well as the crosstalk between neuroinflammation and peripheral inflammatory mediators[38]. The cytokine hypothesis suggests that physiological pathways involved in inflammation and stress responses may play a key role in the pathology of depression. The stress-induced chronic low-grade cytokine release in depression drives the overall immune response toward proinflammation rather than anti-inflammation. The pro-inflammatory mediators in the circulation disrupt both epithelial and endothelial tight junctions, compromising the integrity of the gut-vascular barrier and the BBB[39]. This leads to an increase in intestinal permeability (leaky gut) and a disrupted CNS barrier, providing a gateway for food antigens, toxins, and pathogens from the gut to reach the brain, followed by the activation of neuroimmune cells, which in turn triggers neuroinflammation[39]. For example, the increased systemic inflammation and subsequent neuroinflammation caused by the intraperitoneal injection of bacterial endotoxin LPS mediate microglia activation and neuronal cell death, ultimately leading to cognitive impairment[40]. Indeed, the levels of serum lgA and IgM against the LPS of enterobacteria have been found to be significantly higher in MDD patients than in control subjects[23]. In addition, a study has found elevated expressions of gut microbial 16S rRNA subunit in the blood plasma of MDD patients compared to healthy controls[25].

Despite the conflicting results, there is a general consensus that MDD patients have abnormal levels of pro-inflammatory cytokines in the peripheral circulation[41]. Alvarez-Mon *et al.* have reported that MDD patients have abnormally functioning $CD4^+$ T lymphocytes with increased levels of circulating IL-17 and TNF-α[42]. Pro-inflammatory cytokines can activate the hypothalamic-pituitary-adrenal (HPA) axis in MDD, leading to elevated cortisol levels and glucocorticoid receptor resistance[43]. Furthermore, the pro-inflammatory cytokines released from microglia (*e.g.*, TNF-α) can reduce the levels of neurotransmitters such as serotonin, dopamine, and norepinephrine in MDD patients via several ways, one of which includes reducing their synthesis[44]. Different types of immune cells, such as T cells, produce, store, and respond to serotonin, which is associated with mood disorders[45,46]. In MDD patients, the persistence of pro-inflammatory cytokines (*e.g.*, TNF-α and IL-1β) can impair the BBB function[47]. Under stress conditions, BBB impairments may lead to the infiltration of immune cells. It has been shown that T cells and monocytes infiltrate the brain under stress. In depressed mice models, T helper 17 cells accumulate in different brain regions, such as the hippocampus, promoting depression-like behaviors[48,49].

Byproducts of the gut microbiota can play a neuroimmunomodulatory role in neurological disorders[14]. For example, in a study, the gut microbiome from an aged donor was found to be sufficient to reduce the host SCFAs and induce cognitive decline[50]. SCFAs (primarily acetate, propionate, and butyrate), derived from gut bacterial fermentation of non-digestible carbohydrates, have been reported to be present in human CSF and blood[51]. It is believed that systemic SCFAs can cross the BBB and regulate BBB permeability and microglia functions. For example, a study has found that oral SCFAs can ameliorate depression-like behaviors in mice exposed to chronic stress, which is associated with the inhibition of hippocampal neurogenesis decline, BBB damage, microglia activation, and neuroinflammation by SCFAs[52]. Tryptophan metabolites that are derived from gut microbiota represent another example of the gut-brain axis in the regulation of host inflammatory responses. A study has shown that tryptophan, metabolized by the gut microbiota, can activate aryl hydrocarbon receptor (AhR)

signaling in astrocytes and mitigate CNS inflammation in multiple sclerosis patients[53] as well as improve gut permeability by targeting AhR in a mouse colitis model[54].

Dysregulation of the HPA axis is strongly associated with depressive episodes[55,56]. A direct example of microbiota modulation of HPA responsiveness is the reversibility of the exaggerated HPA stress response in GF mice in response to restraint stress by transplantation of *Bifidobacterium infantis*[57]. GF mice have an underdeveloped immune system due to the absence of commensal microbiota[13,58]. Increased stress reactivity and weakened intestinal barrier have been observed in GF mice[57,59]. Stress is known to induce leaky gut and BBB impairment, thus allowing pathogens and local inflammatory mediators to translocate across the intestinal mucosa and directly affect both immune and neuronal cells in the CNS[19-21,23]. Interestingly, supplementation with *Lactobacillus farciminis* can reduce intestinal permeability and prevent HPA hyperreactivity, induced by restraint stress, in rats[24]. Gut microbiota may regulate brain function through vagus nerve signaling. A study has shown that subdiaphragmatic vagotomy can block depression-like behaviors and reduce the expression of synaptic proteins. This is linked to the increased relative abundance of several beneficial microbiota, including *Lactobacillus* sp. BL302, *Lactobacillus hominis*, and *Lactobacillus reuteri*[60].

3.2. Cellular immune pathway

Intestinal immune cells can directly regulate the neuroimmune balance and neuroinflammatory response. Intestinal B cells differentiate into IgA-secreting plasma cells to regulate the gut microbiota. Gut-derived IgA plasma cells have been found in the CNS of mice with experimental autoimmune encephalomyelitis and to attenuate neuroinflammation in an IL-10-dependent manner[61]. The brain appears to recruit immune cells from the gut to protect the CNS from the entry of peripheral pathogens[61,62]. On the other hand, neuroimmune cells are also modulated by intestinal cells. Subpopulations of IFN-γ-producing meningeal natural killer (NK) cells from the gut can modulate the neuroimmune function and promote astrocyte development; this effect is also regulated by the gut microbiome[63]. Moreover, the depletion of gut microbiome by antibiotics leads to a substantial reduction in IFN-γ-producing meningeal NK cells, downregulating the anti-inflammatory effects of astrocytes, and thus presumably limiting their protective potential[63,64]. Although there is increasing evidence of communication between the gut and the brain, the specific mechanisms are poorly understood. Further studies are needed to reveal the mechanisms by which the gut microbiota and immune responses modulate CNS inflammation in the context of depression.

4. Short-chain fatty acids as key products of gut-brain communication

Endogenous SCFAs are mainly the products of fermentation of non-digestible carbohydrates by gut microbiota. The principal SCFAs in the gut are acetate, propionate, and butyrate, accounting for more than 95% of total SCFAs in the host[65]. SCFAs affect the pathophysiological conditions of the host through a variety of mechanisms, including the regulation of histone acetylation and methylation, activation of G-protein coupled receptors, promotion of hormone (*e.g.*, glucagon-like peptide 1) and neurochemical (*e.g.*, serotonin) secretion, and regulation of vagus nerve circuit[65-67]. Butyrate has been shown to improve cognitive dysfunction in a high-fat diet-induced obese model and a vascular dementia model, while propionate has been reported to reduce the reward response to high-energy food in the human striatum[68-70]. As shown in a study, a mixture of acetate, propionate, and butyrate alleviated alterations in anhedonia and heightened stress responsiveness as well as inhibited increased intestinal permeability induced by psychosocial stress in mice[71].

Receptors (FFARs) that sense and transmit free fatty acid signals have been found in the peripheral and CNSs[72]. Activation of FFARs by SCFAs appears to have a direct and local anti-inflammatory effect on microglia activation[73] as well as a systemic anti-inflammatory effect on neutrophils and dendritic cells that also express FFARs[74]. The ability of SCFAs to cross the BBB and their presence in the brain has been confirmed by injecting radiolabeled ^{14}C-SCFAs into the carotid artery of rats[75]. Neurons and the immune cell crosstalk that contributes to brain function and behavior are affected by SCFAs[76,77]. In addition, SCFAs are important for maintaining the integrity and function of the BBB. It has been shown that propionate, an SCFA, protects the BBB from deleterious inflammatory and oxidative stimuli via transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) signaling[78]. In a study, the increased BBB permeability in GF mice after birth and during adulthood was found to be related to lower expression of BBB tight junction proteins claudin-5 and occludin, which can be mitigated by the exposure of these mice to SCFA-producing bacteria[79]. Reduced SCFA production in autistic patients may lead to gut barrier dysfunction, which could impair immune responses and BBB integrity, contributing to brain dysfunction[80].

A comparison study has demonstrated a decrease in butyrate production (anti-inflammatory properties) by *Faecalibacterium* and an increase in LPS production (pro-inflammatory properties) by *Flavonifractor* in depressed patients compared to healthy controls[81]. A recent systematic review of 26 studies has revealed that patients

with anxiety and depression have a higher abundance of pro-inflammatory bacteria species, such as *Desulfovibrio* and *Enterobacteriaceae*, but lower SCFA-producing *Faecalibacterium* compared to controls[12]. Stilling *et al.* have suggested that butyrate and other SCFAs in the systemic circulation may reduce depression-like behavior in animal models by elevating the levels of histone acetylation and BDNF in specific brain regions, such as the prefrontal cortex[82]. Moreover, sodium butyrate has been shown to ameliorate LPS-induced depression-like behaviors as well as hippocampal microglial inflammation and activation in mice[83].

5. Potential intervention strategies for depression

5.1. Fecal microbiota transplantation (FMT)

Restoring gut homeostasis through FMT may be an effective intervention for depression. Sudo *et al.* have compared GF and specific pathogen-free mice to investigate HPA reaction to stress. GF mice had elevated plasma adrenocorticotropic hormone and corticosterone with reduced BDNF expression in the cortex and hippocampus in response to restraint stress relative to specific pathogen-free (SPF) mice. Interestingly, the exaggerated HPA response in GF mice could be reversed by *B. infantis* or fecal microbes from SPF mice[57]. Recently, Rao *et al.* have demonstrated that FMT increased serotonin expression and decreased the production of IL-1β and TNF-α in a chronic unpredictable stress (CUS) rat model, suppressing the activation of glial cells, and NLRP3 inflammasome in the prefrontal cortex and hippocampus, and thus ameliorating depression-like behaviors[84]. Similarly, our group has found that the transplantation of fecal microbiota into GF mice from NLRP3 KO mice alleviated CUS-induced depression-like behaviors. Moreover, the gut microbiota compositions of NLRP3 KO and wild-type mice differed in the relative abundance of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* spp.[34]. Importantly, Bercik *et al.* have demonstrated that the gut-brain axis is regulated by FMT. GF BALB/c mice that were subjected to microbiota transplantation from the National Institutes of Health (NIH) Swiss mice showed increased exploratory behavior and BDNF levels in the hippocampus. In contrast, GF NIH Swiss mice that were subjected to microbiota transplantation from BALB/c mice had reduced exploratory behavior[85]. Case reports, systematic reviews, and meta-analyses have suggested that FMT can improve depressive symptoms in patients with mental depression[86-89]. These findings strongly suggest that disease or health phenotypes can be transferred from the host to transplanted individuals through the gut microbiota. However, precise and personalized FMT techniques for different diseases (*e.g.*, anxiety, depression, bipolar disorder, and autism spectrum disorders) need to be further investigated.

FMT is considered a promising therapy; for example, FMT has been recommended in clinical guidelines for the treatment of recurrent *Clostridioides difficile* infection[90]. However, FMT has been considered investigational by most regulatory agencies and has not obtained marketing approval from the United State Food and Drug Administration (FDA) for any indication[91]. Although the described risks range from mild gastrointestinal symptoms to severe infection, FMT is largely considered a safe procedure[90,92]. The fact that the variability and uncertainty in the source and donor of fecal microbiota may affect the health risk profile of recipients has been highlighted by recent reports of rare cases of multidrug-resistant organisms, including Shiga toxin-producing *Escherichia coli* and enteropathogenic *E. coli*, where these organisms were not additionally screened[93,94]. While information on the long-term effects of FMT in humans is sparse, several animal studies have shown that disease phenotypes such as obesity and metabolic disorders can be transferred through FMT[95-97]. In the absence of biochemical markers to screen donor stools, there is concern about which phenotypes may escape detection and subsequently be transmitted via FMT. Gupta *et al.* have examined the evolving risk of FMT and affirmed that screening for such organisms or markers has been a standard practice since 2016[90,98]. Therefore, much work remains to be done before FMT can be applied to the clinical management of depression. One alternative to FMT is dietary therapy, such as increasing the abundance of probiotics in the gut through consistent consumption of probiotics, which will be further discussed in the next section.

5.2. Dietary interventions for depression

There is a growing interest in the relationship between dietary patterns and depression. Emerging evidence has suggested that diet has a strong influence on neurological processes and emotional behavior because it strongly affects the gut microbiota[99,100]. Long-term anti-inflammatory dietary patterns may prevent depression, whereas pro-inflammatory patterns may promote depression-related conditions[100,101]. However, the mechanisms underlying this relationship are currently unclear. A "Western" dietary pattern, which is characterized by high consumption of sweet and fatty food, fried and processed food, refined grains, red meat, and high-fat dairy products, with low consumption of fruits and vegetables, is associated with a higher incidence of depression[102-104]. In a randomized controlled trial, a Mediterranean-style dietary pattern supplemented with fish oil has shown to improve the mental

health of patients with depression[103]. A health survey that included 521 municipal employees (309 men and 212 women) aged 21 – 67 years has shown an association between less depressive symptoms and a healthy Japanese dietary pattern, consisting of a high intake of vegetables, fruits, mushrooms, and soy products[105]. A healthy dietary pattern is characterized by a high intake of vegetables, fruits, nuts, grains, seeds, and legumes, as well as a moderate consumption of dairy products, eggs, and fish. Investigations have shown that a number of nutrients, including magnesium, zinc, folate, Vitamin D, Vitamin B_{12}, Vitamin B_6, and Vitamin E, which are all abundant in fruits and vegetables, are associated with reduced risk of depression[106-108]. These nutrients can modulate depressive symptoms through various mechanisms, two of which include regulating serotonin biosynthesis and the HPA axis as well as inhibiting glutamate signaling, inflammation, and oxidative stress[109,110].

Meta-analyses have shown that omega-3 polyunsaturated fatty acids (particularly eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) have a beneficial effect on mood in patients with MDD[111,112]. A study has shown that the long-term supplementation of omega-3 polyunsaturated fatty acids (80% EPA plus 20% DHA) restored the disturbed gut microbiota composition in early-life stress (maternal separation)-treated rats[113]. A deficiency of omega-3 can substantially impair CNS development, with symptoms first appearing as early as adolescence, such as decreased sociability and increased depressive-like behaviors. An increase in abundance of *Firmicutes* and a decrease in abundance of *Bacteroidetes* in fecal microbiota have been observed in omega-3 deficiency mice during adolescence. Omega-3 supplementation has shown to induce a higher abundance of *Bifidobacterium* and *Lactobacillus* but a lower abundance of *Anaeroplasma*, *Clostridium*, and *Peptostreptococcaceae* in adult mice[114]. Grosso *et al.* have reviewed other evidence and mechanisms of omega-3 in depression, including the modulation of serotoninergic and dopaminergic transmission as well as the inhibition of chronic systemic inflammation by omega-3[115].

Healthy dietary patterns promote a higher growth of gut probiotics. For example, prebiotics from healthy dietary patterns selectively promote the growth and activity of *Bifidobacteria* and *Lactobacilli*, conferring positive health outcomes to the host[66]. The most well-studied prebiotics are fructo-oligosaccharides (FOS), inulin, and galacto-oligosaccharides (GOS)[66]. GOS feeding has shown to elevate BDNF and D-serine expression and N-methyl-D-aspartate pathway in the hippocampus as well as increase the levels of gut peptide YY in rat plasma[116]. In a clinical study with human subjects, an anxiolytic-like effect was observed with GOS supplementation, as evidenced by decreased waking salivary cortisol reactivity and altered attentional vigilance compared to placebo[117]. The cortisol arousal response, which can increase under stress, is a marker of HPA axis activity[118,119]. A mixture of GOS and FOS has shown to reduce corticosterone and pro-inflammatory cytokine release as well as anxiety-like and depressive-like behaviors in chronic stress-treated mice. These benefits may be associated with increased cecal acetate and propionate and reduced butyrate concentrations by GOS and FOS administration[120]. A mixture of GOS and polydextrose has shown to attenuate the negative scores of memory learning and anxiety-like behavior in an early-life maternal separation rat model, while a combined supplementation (*Lactobacillus rhamnosus* GG, polydextrose, and GOS) can enhance these benefits[121]. In another study, a synbiotic supplementation containing both prebiotics (GOS, FOS, and inulin) and probiotics (*Lactobacillus acidophilus* T16, *Bifidobacterium bifidum* BIA-6, *Bifidobacterium lactis* BIA-7, and *Bifidobacterium longum* BIA-8) was given to patients undergoing hemodialysis for 12 weeks; it resulted in a significant improvement in serum BDNF concentrations and depressive symptoms compared to probiotic supplementation alone[122]. The systemic and neurological benefits induced by an increased growth of probiotics in the gut and the subsequently elevated levels of SCFAs in the circulation appear to be a possible explanation. SCFAs have been reported to promote hippocampal neurogenesis; suppress BBB damage, microglia activation, and neuroinflammation; as well as ameliorate depressive-like behaviors in high fructose diet-fed mice exposed to chronic stress[52]. Marx *et al.*[123] and Bear *et al.*[99] have provided interesting reviews of the underlying mechanisms of action by which diet may influence mental and brain health.

However, not all studies show an association between depression and dietary interventions. In a large, randomized trial of 18,353 adults with 16,657 at risk of incident depression (no previous depression) and 1696 at risk of recurrent depression, omega-3 supplements were not recommended in adults to prevent depression. Compared to placebo treatment, omega-3 treatment increased the risk of incident depression, but not recurrent depression[124]. A systematic review and meta-analysis of 28 controlled clinical trials have shown that probiotics, instead of prebiotics, yielded small but significant effects on depression and anxiety[125]. The conflicting findings from different studies may be due to various factors. Subjects may have recall bias when scales are used. In addition, depression can affect memory, food intake activity, and food preferences in subjects. It is difficult to ensure that

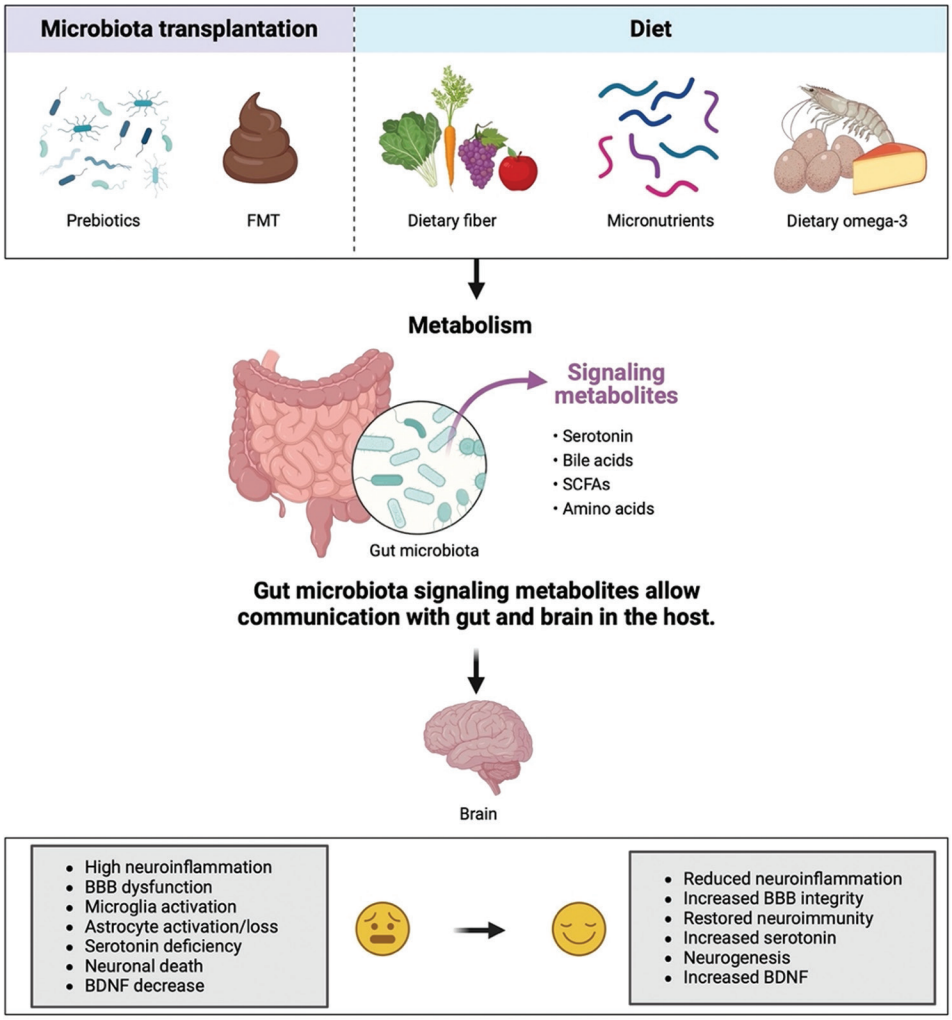


Figure 2. Potential intervention strategies for depression via the gut-brain axis. Emerging interventions targeting the gut-brain axis, such as prebiotics, FMT, healthy dietary patterns, omega-3, and other micronutrients, are providing insights into the prevention and treatment of depression. A healthy composition of gut microbiota directly or indirectly induces the production of beneficial metabolites, including serotonin, bile acids, SCFAs, and amino acids, which facilitate communication between the gut and the brain. These beneficial metabolites modulate the brain from a "sad" pro-inflammatory state to a "happy" anti-inflammatory state.

BBB: Blood-brain barrier; BDNF: Brain-derived neurotrophic factor; FMT: Fecal microbiome transplantation; SCFAs: Short-chain fatty acids. Created with BioRender.com.

the subjects remain oblivious to the study hypothesis and content in addition to controlling all confounding variables.

6. Conclusion and future perspectives

The role of gut microbiota in depression has garnered widespread attention. The gut-brain axis is an interactive system that involves complex networks of neural, chemical, inflammatory, and immune interactions. This review describes some interesting research focus and pathophysiology of gut-brain interactions in depression, including serotonin, HPA axis, neuroinflammation, gut homeostasis, and SCFAs. Depression is characterized by low-grade systemic and neurological inflammation, leakage of the BBB and intestinal mucosal barrier, a high relative abundance of the pro-inflammatory species of gut microbiota, and a low abundance of SCFA-producing probiotics (Figure 1). Based on this, FMT and healthy dietary interventions are considered to be promising therapeutic strategies for depression (Figure 2).

Nevertheless, there are still many issues that need to be addressed in the future. Although several hypotheses have been reported, including pro-inflammatory mediators, oxidative stress, and endotoxin translocation, the

mechanisms by which stress triggers leaky gut and BBB damage remain unclear. Changes in the composition of gut microbiota have been observed in animal models and also in patients with depression, but the causal relationship between bacterial dysbiosis and depression remains ambiguous. Although FMT and dietary therapies targeting gut homeostasis hold great promise for the treatment of depression, the conflicting results and limited research on the former have delayed clinical translation and application. Further studies are needed to resolve the contribution of potential confounders to these interactions.

FMT and dietary interventions may have a dual benefit: first, by directly repairing the gut damage and inflammatory state of the host; and second, by indirectly modulating the gut microbiota and metabolism. More research on the mechanisms of the gut-lung axis in both humans and animals will continue to contribute to the understanding of the bidirectional effects of neuroinflammation and gut microbiota in depression as well as in other psychological and neurological diseases. Consequently, it is anticipated that a well-understood and defined individualized program to treat and prevent depression will be developed.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Ethics approval and consent to participate

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References

1. Global Health Data Exchange, 2019, *Institute of Health Metrics and Evaluation*. Available from: https://www.vizhub.healthdata.org/gbd-results [Last accesed on 2022 Oct 14].

2. World Health Organization, 2022, *Mental Health and COVID-19: Early Evidence of the Pandemic's Impact*. Geneva: World Health Organization. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-Sci_Brief-Mental_health-2022.1 [Last accessed on 2022 Oct 15].

3. World Health Organization, 2022, *Depression: Fact Sheets*. Available from: https://www.who.int/news-room/fact-sheets/detail/depression [Last accessed on 2022 Oct 14].

4. DeRubeis RJ, Siegle GJ, Hollon SD, 2008, Cognitive therapy versus medication for depression: Treatment outcomes and neural mechanisms. *Nat Rev Neurosci*, 9: 788–796.

   https://doi.org/10.1038/nrn2345

5. Heneka MT, Carson MJ, El Khoury J, *et al.*, 2015, Neuroinflammation in Alzheimer's disease. *Lancet Neurol*, 14: 388–405.

   https://doi.org/10.1016/S1474-4422(15)70016-5

6. De Virgilio A, Greco A, Fabbrini G, *et al.*, 2016, Parkinson's disease: Autoimmunity and neuroinflammation. *Autoimmun Rev*, 15: 1005–1111.

   https://doi.org/10.1016/j.autrev.2016.07.022

7. Troubat R, Barone P, Leman S, *et al.*, 2021, Neuroinflammation and depression: A review. *Eur J Neurosci*, 53: 151–171.

   https://doi.org/10.1111/ejn.14720

8. Raison CL, Capuron L, Miller AH, 2006, Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol*, 27: 24–31.

   https://doi.org/10.1016/j.it.2005.11.006

9. Musselman DL, Lawson DH, Gumnick JF, *et al.*, 2001, Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N Engl J Med*, 344: 961–966.

   https://doi.org/10.1056/NEJM200103293441303

10. Haapakoski R, Mathieu J, Ebmeier KP, *et al.*, 2015, Cumulative meta-analysis of interleukins 6 and 1beta, tumour necrosis factor alpha and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun*, 49: 206–215.

    https://doi.org/10.1016/j.bbi.2015.06.001

11. Kappelmann N, Lewis G, Dantzer R, *et al.*, 2018, Antidepressant activity of anti-cytokine treatment: A systematic review and meta-analysis of clinical trials of chronic inflammatory conditions. *Mol Psychiatry*, 23: 335–343.

    https://doi.org/10.1038/mp.2016.167

12. Simpson CA, Diaz-Arteche C, Eliby D, *et al.*, 2021, The gut microbiota in anxiety and depression- A systematic review. *Clin Psychol Rev*, 83: 101943.

https://doi.org/10.1016/j.cpr.2020.101943

13. Foster JA, Neufeld KA, 2013, Gut-brain axis: How the microbiome influences anxiety and depression. *Trends Neurosci*, 36: 305–312.

https://doi.org/10.1016/j.tins.2013.01.005

14. Cryan JF, O'Riordan KJ, Cowan CS, *et al.*, 2019, The microbiota-gut-brain axis. *Physiol Rev*, 99: 1877–2013.

https://doi.org/10.1152/physrev.00018.2018

15. Valles-Colomer M, Falony G, Darzi Y, *et al.*, 2019, The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat Microbiol*, 4: 623–632.

https://doi.org/10.1038/s41564-018-0337-x

16. Jiang H, Ling Z, Zhang Y, *et al.*, 2015, Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun*, 48: 186–194.

https://doi.org/10.1016/j.bbi.2015.03.016

17. Yang Z, Li J, Gui X, *et al.*, 2020, Updated review of research on the gut microbiota and their relation to depression in animals and human beings. *Mol Psychiatry*, 25: 2759–2772.

https://doi.org/10.1038/s41380-020-0729-1

18. Messaoudi M, Lalonde R, Violle N, *et al.*, 2011, Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br J Nutr*, 105: 755–764.

https://doi.org/10.1017/S0007114510004319

19. Camilleri M, 2019, Leaky gut: Mechanisms, measurement and clinical implications in humans. *Gut*, 68: 1516–1526.

https://doi.org/10.1136/gutjnl-2019-318427

20. Ohlsson L, Gustafsson A, Lavant E, *et al.*, 2019, Leaky gut biomarkers in depression and suicidal behavior. *Acta Psychiatr Scand*, 139: 185–193.

https://doi.org/10.1111/acps.12978

21. Vanuytsel T, van Wanrooy S, Vanheel H, *et al.*, 2014, Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. *Gut*, 63: 1293–1299.

https://doi.org/10.1136/gutjnl-2013-305690

22. Clark A, Mach, 2016, Exercise-induced stress behavior, gut-microbiota-brain axis and diet: A systematic review for athletes. *J Int Soc Sports Nutr*, 13: 43.

https://doi.org/10.1186/s12970-016-0155-6

23. Maes M, Kubera M, Leunis JC, 2008, The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuro Endocrinol Lett*, 29: 117–124.

24. Ait-Belgnaoui A, Durand H, Cartier C, *et al.*, 2012, Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. *Psychoneuroendocrinology*, 37: 1885–1895.

https://doi.org/10.1016/j.psyneuen.2012.03.024

25. Keri S, Szabo C, Kelemen O, 2014, Expression of toll-like receptors in peripheral blood mononuclear cells and response to cognitive-behavioral therapy in major depressive disorder. *Brain Behav Immun*, 40: 235–243.

https://doi.org/10.1016/j.bbi.2014.03.020

26. Eckburg PB, Bik EM, Bernstein CN, *et al.*, 2005, Diversity of the human intestinal microbial flora. *Science*, 308: 1635–1638.

https://doi.org/10.1126/science.1110591

27. Zheng P, Yang J, Li Y, *et al.*, 2020, Gut microbial signatures can discriminate unipolar from bipolar depression. *Adv Sci* (*Weinh*), 7: 1902862.

https://doi.org/10.1002/advs.201902862

28. Zheng P, Zeng B, Zhou C, *et al.*, 2016, Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol Psychiatry*, 21: 786–796.

https://doi.org/10.1038/mp.2016.44

29. Yang J, Zheng P, Li Y, *et al.*, 2020, Landscapes of bacterial and metabolic signatures and their interaction in major depressive disorders. *Sci Adv*, 6: eaba8555.

https://doi.org/10.1126/sciadv.aba8555

30. Lin CH, Chen CC, Chiang HL, *et al.*, 2019, Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. *J Neuroinflammation*, 16: 129.

https://doi.org/10.1186/s12974-019-1528-y

31. Bajaj JS, Hylemon PB, Ridlon JM, *et al.*, 2012, Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. *Am J Physiol Gastrointest Liver Physiol*, 303: G675–G685.

https://doi.org/10.1152/ajpgi.00152.2012

32. Pu Y, Zhang Q, Tang Z, *et al.*, 2022, Fecal microbiota transplantation from patients with rheumatoid arthritis causes depression-like behaviors in mice through abnormal T cells activation. *Transl Psychiatry*, 12: 223.

https://doi.org/10.1038/s41398-022-01993-z

33. Kelly JR, Borre Y, O'Brien C, *et al.*, 2016, Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *J Psychiatr Res*, 82: 109–118.

https://doi.org/10.1016/j.jpsychires.2016.07.019

34. Zhang Y, Huang R, Cheng M, *et al.*, 2019, Gut microbiota from NLRP3-deficient mice ameliorates depressive-like behaviors by regulating astrocyte dysfunction via circHIPK2. *Microbiome*, 7: 116.

https://doi.org/10.1186/s40168-019-0733-3

35. McGuinness AJ, Davis JA, Dawson SL, *et al.*, 2022, A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. *Mol Psychiatry*, 27: 1920–1935.

http://doi.org/10.1038/s41380-022-01456-3

36. Fung TC, Olson CA, Hsiao EY, 2017, Interactions between the microbiota, immune and nervous systems in health and disease. *Nat Neurosci*, 20: 145–155.

https://doi.org/10.1038/nn.4476

37. Rutsch A, Kantsjo JB, Ronchi F, 2020, The gut-brain axis: How microbiota and host inflammasome influence brain physiology and pathology. *Front Immunol*, 11: 604179.

https://doi.org/10.3389/fimmu.2020.604179

38. Carlessi AS, Borba LA, Zugno AI, *et al.*, 2021, Gut microbiota-brain axis in depression: The role of neuroinflammation. *Eur J Neurosci*, 53: 222–235.

https://doi.org/10.1111/ejn.14631

39. Parker A, Fonseca S, Carding SR, 2020, Gut microbes and metabolites as modulators of blood-brain barrier integrity and brain health. *Gut Microbes*, 11: 135–157.

https://doi.org/10.1080/19490976.2019.1638722

40. Zhao J, Bi W, Xiao S, *et al.*, 2019, Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. *Sci Rep*, 9: 5790.

https://doi.org/10.1038/s41598-019-42286-8

41. Kohler CA, Freitas TH, Maes M, *et al.*, 2017, Peripheral cytokine and chemokine alterations in depression: A meta-analysis of 82 studies. *Acta Psychiatr Scand*, 135: 373–387.

https://doi.org/10.1111/acps.12698

42. Alvarez-Mon MA, Gomez-Lahoz AM, Orozco A, *et al.*, 2021, Expansion of CD4 T lymphocytes expressing interleukin 17 and tumor necrosis factor in patients with major depressive disorder. *J Pers Med*, 11: 220.

https://doi.org/10.3390/jpm11030220

43. Zunszain PA, Anacker C, Cattaneo A, *et al.*, 2011, Glucocorticoids, cytokines and brain abnormalities in depression. *Prog Neuropsychopharmacol Biol Psychiatry*, 35: 722–729.

https://doi.org/10.1016/j.pnpbp.2010.04.011

44. Miller AH, Raison CL, 2016, The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol*, 16: 22–34.

https://doi.org/10.1038/nri.2015.5

45. Wu H, Denna TH, Storkersen JN, *et al.*, 2019, Beyond a neurotransmitter: The role of serotonin in inflammation and immunity. *Pharmacol Res*, 140: 100–114.

https://doi.org/10.1016/j.phrs.2018.06.015

46. Correia AS, Cardoso A, Vale N, 2021, Highlighting immune system and stress in major depressive disorder, Parkinson's, and Alzheimer's Diseases, with a connection with serotonin. *Int J Mol Sci*, 22: 8525.

https://doi.org/10.3390/ijms22168525

47. Niklasson F, Agren H, 1984, Brain energy metabolism and blood-brain barrier permeability in depressive patients: Analyses of creatine, creatinine, urate, and albumin in CSF and blood. *Biol Psychiatry*, 19: 1183–1206.

48. Beurel E, Lowell JA, Jope RS, 2018, Distinct characteristics of hippocampal pathogenic T(H)17 cells in a mouse model of depression. *Brain Behav Immun*, 73: 180–191.

https://doi.org/10.1016/j.bbi.2018.04.012

49. Beurel E, Harrington LE, Jope RS, 2013, Inflammatory T helper 17 cells promote depression-like behavior in mice. *Biol Psychiatry*, 73: 622–630.

https://doi.org/10.1016/j.biopsych.2012.09.021

50. Lee J, Venna VR, Durgan DJ, *et al.*, 2020, Young versus aged microbiota transplants to germ-free mice: Increased short-chain fatty acids and improved cognitive performance. *Gut Microbes*, 12: 1–14.

https://doi.org/10.1080/19490976.2020.1814107

51. O'Riordan KJ, Collins MK, Moloney GM, *et al.*, 2022, Short chain fatty acids: Microbial metabolites for gut-brain axis signalling. *Mol Cell Endocrinol*, 546: 111572.

https://doi.org/10.1016/j.mce.2022.111572

52. Tang CF, Wang CY, Wang JH, *et al.*, Short-chain fatty acids ameliorate depressive-like behaviors of high fructose-fed mice by rescuing hippocampal neurogenesis decline and blood-brain barrier damage. *Nutrients*, 14: 1882.

https://doi.org/10.3390/nu14091882

53. Rothhammer V, Mascanfroni ID, Bunse L, *et al.*, 2016, Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat Med*, 22: 586–957.

https://doi.org/10.1038/nm.4106

54. Scott SA, Fu J, Chang PV, 2020, Microbial tryptophan metabolites regulate gut barrier function via the aryl hydrocarbon receptor. *Proc Natl Acad Sci U S A*, 117: 19376–19387.

https://doi.org/10.1073/pnas.2000047117

55. Barden N, 2004, Implication of the hypothalamic-pituitary-adrenal axis in the physiopathology of depression.

J Psychiatry Neurosci, 29: 185–193.

56. Wang X, Li Y, Wu L, *et al.*, 2021, Dysregulation of the gut-brain-skin axis and key overlapping inflammatory and immune mechanisms of psoriasis and depression. *Biomed Pharmacother*, 137: 111065.

https://doi.org/10.1016/j.biopha.2020.111065

57. Sudo N, Chida Y, Aiba Y, *et al.*, 2004, Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol*, 558: 263–275.

https://doi.org/10.1113/jphysiol.2004.063388

58. Luckey TD, 1965, Effects of microbes on germfree animals. *Adv Appl Microbiol*, 7: 169–223.

https://doi.org/10.1016/s0065-2164(08)70387-3

59. Hernandez-Chirlaque C, Aranda CH, Ocon B, *et al.*, 2016, Germ-free and antibiotic-treated mice are highly susceptible to epithelial injury in DSS colitis. *J Crohns Colitis*, 10: 1324–1335.

https://doi.org/10.1093/ecco-jcc/jjw096

60. Yang Y, Eguchi A, Wan X, *et al.*, 2023, A role of gut-microbiota-brain axis via subdiaphragmatic vagus nerve in depression-like phenotypes in Chrna7 knock-out mice. *Prog Neuropsychopharmacol Biol Psychiatry*, 120: 110652.

https://doi.org/10.1016/j.pnpbp.2022.110652

61. Rojas OL, Probstel Ak, Porfilio EA, *et al.*, 2019, Recirculating intestinal IgA-producing cells regulate neuroinflammation via IL-10. *Cell*, 176: 610–624.e18.

https://doi.org/10.1016/j.cell.2018.11.035

62. Fitzpatrick Z, Frazer G, Ferro A, *et al.*, 2020, Gut-educated IgA plasma cells defend the meningeal venous sinuses. *Nature*, 587: 472–476.

https://doi.org/10.1038/s41586-020-2886-4

63. Sanmarco LM, Wheeler MA, Gutierrez-Vazquez C, *et al.*, 2021, Gut-licensed IFNgamma(+) NK cells drive LAMP1(+) TRAIL(+) anti-inflammatory astrocytes. *Nature*, 590: 473–479.

https://doi.org/10.1038/s41586-020-03116-4

64. Verkhratsky A, Illes P, Tang Y, *et al.*, 2021, The anti-inflammatory astrocyte revealed: The role of the microbiome in shaping brain defences. *Signal Transduct Target Ther*, 6: 150.

https://doi.org/10.1038/s41392-021-00577-5

65. Koh A, De Vadder F, Kovatcheva-Datchary P, *et al.*, 2016, From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell*, 165: 1332–1345.

https://doi.org/10.1016/j.cell.2016.05.041

66. Cai Y, Folkerts J, Folkerts G, *et al.*, 2020, Microbiota-dependent and -independent effects of dietary fibre on human health. *Br J Pharmacol*, 177: 1363–1381.

https://doi.org/10.1111/bph.14871

67. Li Z, Yi CX, Katiraei S, *et al.*, 2018, Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. *Gut*, 67: 1269–1279.

https://doi.org/10.1136/gutjnl-2017-314050

68. Arnoldussen IAC, Wiesmann M, Pelgrim CE, *et al.*, 2017, Butyrate restores HFD-induced adaptations in brain function and metabolism in mid-adult obese mice. *Int J Obes* (*Lond*), 41: 935–944.

https://doi.org/10.1038/ijo.2017.52

69. Liu J, Sun J, Wang F, *et al.*, 2015, Neuroprotective effects of clostridium butyricum against vascular dementia in mice via metabolic butyrate. *Biomed Res Int*, 2015: 412946.

https://doi.org/10.1155/2015/412946

70. Byrne CS, Chambers ES, Alhabeeb H, *et al.*, 2016, Increased colonic propionate reduces anticipatory reward responses in the human striatum to high-energy foods. *Am J Clin Nutr*, 104: 5–14.

https://doi.org/10.3945/ajcn.115.126706

71. van de Wouw M, Boehme M, Lyte JM, *et al.*, 2018, Short-chain fatty acids: Microbial metabolites that alleviate stress-induced brain-gut axis alterations. *J Physiol*, 596: 4923–4944.

https://doi.org/10.1113/JP276431

72. Falomir-Lockhart LJ, Cavazzutti GF, Gimenez E, *et al.*, Fatty acid signaling mechanisms in neural cells: Fatty acid receptors. *Front Cell Neurosci*, 13: 162.

https://doi.org/10.3389/fncel.2019.00162

73. Erny D, de Angelis AL, Jaitin D, *et al.*, 2015, Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci*, 18: 965–977.

https://doi.org/10.1038/nn.4030

74. Maslowski KM, Vieira AT, Ng A, *et al.*, 2009, Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*, 461: 1282–1286.

https://doi.org/10.1038/nature08530

75. Oldendorf WH, 1973, Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. *Am J Physiol*, 224: 1450–1453.

https://doi.org/10.1152/ajplegacy.1973.224.6.1450

76. Agirman G, Hsiao EY, 2021, SnapShot: The microbiota-gut-brain axis. *Cell*, 184: 2524–2524.e1.

https://doi.org/10.1016/j.cell.2021.03.022

77. Xiao S, Jiang S, Qian D, *et al.*, 2020, Modulation of microbially derived short-chain fatty acids on intestinal homeostasis, metabolism, and neuropsychiatric disorder. *Appl Microbiol Biotechnol*, 104: 589–601.

https://doi.org/10.1007/s00253-019-10312-4

78. Hoyles L, Snelling T, Umlai UK, *et al.*, 2018, Microbiome-host systems interactions: Protective effects of propionate upon the blood-brain barrier. *Microbiome*, 6: 55.

https://doi.org/10.1186/s40168-018-0439-y

79. Braniste V, Al-Asmakh M, Kowal C, *et al.*, 2014, The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med*, 6: 263ra158.

https://doi.org/10.1126/scitranslmed.3009759

80. Morris G, Berk M, Carvalho A, *et al.*, 2017, The role of the microbial metabolites including tryptophan catabolites and short chain fatty acids in the pathophysiology of immune-inflammatory and neuroimmune disease. *Mol Neurobiol*, 54: 4432–4451.

https://doi.org/10.1007/s12035-016-0004-2

81. Liu RT, Rowan-Nash AD, Sheehan AE, *et al.*, 2020, Reductions in anti-inflammatory gut bacteria are associated with depression in a sample of young adults. *Brain Behav Immun*, 88: 308–324.

https://doi.org/10.1016/j.bbi.2020.03.026

82. Stilling RM, van de Wouw M, Clarke G, *et al.*, 2016, The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis? *Neurochem Int*, 99: 110–132.

https://doi.org/10.1016/j.neuint.2016.06.011

83. Yamawaki Y, Yoshioka N, Nozaki K, *et al.*, 2018, Sodium butyrate abolishes lipopolysaccharide-induced depression-like behaviors and hippocampal microglial activation in mice. *Brain Res*, 1680: 13–38.

https://doi.org/10.1016/j.brainres.2017.12.004

84. Rao J, Qiao Y, Xie R, *et al.*, 2021, Fecal microbiota transplantation ameliorates stress-induced depression-like behaviors associated with the inhibition of glial and NLRP3 inflammasome in rat brain. *J Psychiatr Res*, 137: 147–157.

https://doi.org/10.1016/j.jpsychires.2021.02.057

85. Bercik P, Denou E, Collins J, *et al.*, 2011, The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, 141: 599–609, 609 e1–3.

https://doi.org/10.1053/j.gastro.2011.04.052

86. Cai T, Shi X, Yuan LZ, *et al.*, 2019, Fecal microbiota transplantation in an elderly patient with mental depression. *Int Psychogeriatr*, 31: 1525–1526.

http://doi.org/10.1017/S1041610219000115

87. Meyyappan AC, Forth E, Wallace CJK, *et al.*, 2020, Effect of fecal microbiota transplant on symptoms of psychiatric disorders: A systematic review. *BMC Psychiatry*, 20: 299.

https://doi.org/10.1186/s12888-020-02654-5

88. Fond GB, Lagier JC, Honore S, *et al.*, 2020, Microbiota-orientated treatments for major depression and schizophrenia. *Nutrients*, 12: 1024.

https://doi.org/10.3390/nu12041024

89. Green JE, Berk M, Loughman A, *et al.*, 2021, FMT for psychiatric disorders: Following the brown brick road into the future. *Bipolar Disord*, 23: 651–655.

https://doi.org/10.1111/bdi.13124

90. Gupta S, Mullish BH, Allegretti JR, 2021, Fecal microbiota transplantation: The evolving risk landscape. *Am J Gastroenterol*, 116: 647–656.

http://doi.org/10.14309/ajg.0000000000001075

91. Grigoryan Z, Shen MJ, Twardus SW, *et al.*, 2020, Fecal microbiota transplantation: Uses, questions, and ethics. *Med Microecol*, 6: 100027.

https://doi.org/10.1016/j.medmic.2020.100027

92. Wang S, Xu M, Wang W, *et al.*, 2016, Systematic review: Adverse events of fecal microbiota transplantation. *PLoS One*, 11: e0161174.

https://doi.org/10.1371/journal.pone.0161174

93. DeFilipp Z, Bloom PP, Soto MT, *et al.*, 2019, Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med*, 381: 2043–2050.

https://doi.org/10.1056/NEJMoa1910437

94. Zellmer C, Sater MR, Huntley MH, *et al.*, 2021, Shiga toxin-producing *Escherichia coli* transmission via fecal microbiota transplant. *Clin Infect Dis*, 72: e876–e880.

https://doi.org/10.1093/cid/ciaa1486

95. Vrieze A, Van Nood E, Holleman F, *et al.*, 2012, Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*, 143: 913–916.e7.

https://doi.org/10.1053/j.gastro.2012.06.031

96. Ridaura VK, Faith JJ, Rey FE, *et al.*, 2013, Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*, 341: 1241214.

https://doi.org/10.1126/science.1241214

97. Vijay-Kumar M, Aitken JD, Carvalho FA, *et al.*, 2010, Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science*, 328: 228–231.

https://doi.org/10.1126/science.1179721

98. Kassam Z, Dubois N, Ramakrishna B, *et al.*, 2019, Donor screening for fecal microbiota transplantation. *N Engl J Med*, 381: 2070–2072.

https://doi.org/10.1056/NEJMc1913670

99. Bear TLK, Dalziel JE, Coad J, *et al.*, 2020, The role of the gut microbiota in dietary interventions for depression and anxiety. *Adv Nutr*, 11: 890–907.

https://doi.org/10.1093/advances/nmaa016

100. Li X, Chen M, Yao Z, *et al.*, 2022, Dietary inflammatory potential and the incidence of depression and anxiety: A meta-analysis. *J Health Popul Nutr*, 41: 24.

https://doi.org/10.1186/s41043-022-00303-z

101. Chen GQ, Peng CL, Lian Y, *et al.*, 2021, Association between dietary inflammatory index and mental health: A systematic review and dose-response meta-analysis. *Front Nutr*, 8: 662357.

https://doi.org/10.3389/fnut.2021.662357

102. Ruusunen A, Lehto SM, Mursu J, *et al.*, 2014, Dietary patterns are associated with the prevalence of elevated depressive symptoms and the risk of getting a hospital discharge diagnosis of depression in middle-aged or older Finnish men. *J Affect Disord*, 159: 1–6.

https://doi.org/10.1016/j.jad.2014.01.020

103. Parletta N, Zarnowiecki D, Cho J, *et al.*, 2019, A mediterranean-style dietary intervention supplemented with fish oil improves diet quality and mental health in people with depression: A randomized controlled trial (HELFIMED). *Nutr Neurosci*, 22: 474–487.

https://doi.org/10.1080/1028415X.2017.1411320

104. Akbaraly TN, Brunner EJ, Ferrie JE, *et al.*, 2009, Dietary pattern and depressive symptoms in middle age. *Br J Psychiatry*, 195: 408–413.

https://doi.org/10.1192/bjp.bp.108.058925

105. Nanri A, Kimura Y, Matsushita Y, *et al.*, 2020, Dietary patterns and depressive symptoms among Japanese men and women. *Eur J Clin Nutr*, 64: 832–839.

https://doi.org/10.1038/ejcn.2010.86

106. Gougeon L, Payette H, Morais JA, *et al.*, 2016, Intakes of folate, Vitamin B6 and B12 and risk of depression in community-dwelling older adults: The quebec longitudinal study on nutrition and aging. *Eur J Clin Nutr*, 70: 380–385.

https://doi.org/10.1038/ejcn.2015.202

107. Anglin RE, Samaan Z, Walter SD, *et al.*, 2013, Vitamin D deficiency and depression in adults: Systematic review and meta-analysis. *Br J Psychiatry*, 202: 100–107.

https://doi.org/10.1192/bjp.bp.111.106666

108. Jacka FN, Maes M, Pasco JA, *et al.*, 2012, Nutrient intakes and the common mental disorders in women. *J Affect Disord*, 141: 79–85.

https://doi.org/10.1016/j.jad.2012.02.018

109. Miller AL, 2008, The methylation, neurotransmitter, and antioxidant connections between folate and depression. *Altern Med Rev*, 13: 216–226.

110. Wang J, Um P, Dickerman BA, *et al.*, 2018, Zinc, magnesium, selenium and depression: A review of the evidence, potential mechanisms and implications. *Nutrients*, 10: 584.

https://doi.org/10.3390/nu10050584

111. Grosso G, Pajak A, Marventano S, *et al.*, 2014, Role of omega-3 fatty acids in the treatment of depressive disorders: A comprehensive meta-analysis of randomized clinical trials. *PLoS One*, 9: e96905.

https://doi.org/10.1371/journal.pone.0096905

112. Appleton KM, Rogers PJ, Ness AR, 2010, Updated systematic review and meta-analysis of the effects of n-3 long-chain polyunsaturated fatty acids on depressed mood. *Am J Clin Nutr*, 91: 757–770.

https://doi.org/10.3945/ajcn.2009.28313

113. Pusceddu MM, El Aidy S, Crispie F, *et al.*, 2015, N-3 Polyunsaturated fatty acids (PUFAs) reverse the impact of early-life stress on the gut microbiota. *PLoS One*, 10: e0139721.

https://doi.org/10.1371/journal.pone.0139721

114. Robertson RC, Oriach CS, Murphy K, *et al.*, 2017, Omega-3 polyunsaturated fatty acids critically regulate behaviour and gut microbiota development in adolescence and adulthood. *Brain Behav Immun*, 59: 21–37.

https://doi.org/10.1016/j.bbi.2016.07.145

115. Grosso G, Galvano F, Marventano S, *et al.*, 2014, Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. *Oxid Med Cell Longev*, 2014: 313570.

https://doi.org/10.1155/2014/313570

116. Savignac HM, Corona G, Mills H, *et al.*, 2013, Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-D-aspartate receptor subunits and D-serine. *Neurochem Int*, 63: 756–764.

https://doi.org/10.1016/j.neuint.2013.10.006

117. Schmidt K, Cowen PJ, Harmer CJ, *et al.*, 2015, Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology* (*Berl*), 232: 1793–1801.

https://doi.org/10.1007/s00213-014-3810-0

118. Kunz-Ebrecht SR, Kirschbaum C, Marmot M, *et al.*, 2004, Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology*, 29: 516–528.

https://doi.org/10.1016/s0306-4530(03)00072-6

119. Pruessner JC, Wolf OT, Hellhammer DH, *et al.*, 1997, Free cortisol levels after awakening: A reliable biological marker for the assessment of adrenocortical activity. *Life Sci*, 61: 2539–2549.

https://doi.org/10.1016/s0024-3205(97)01008-4

120. Burokas A, Arboleya S, Moloney RD, *et al.*, 2017, Targeting the microbiota-gut-brain axis: Prebiotics have anxiolytic and antidepressant-like effects and reverse the impact of

chronic stress in mice. *Biol Psychiatry*, 82: 472–487.

https://doi.org/10.1016/j.biopsych.2016.12.031

121. Neufeld KA, O'Mahony SM, Hoban AE, *et al.*, 2019, Neurobehavioural effects of *Lactobacillus rhamnosus* GG alone and in combination with prebiotics polydextrose and galactooligosaccharide in male rats exposed to early-life stress. *Nutr Neurosci*, 22: 425–434.

https://doi.org/10.1080/1028415X.2017.1397875

122. Haghighat N, Rajabi S, Mohammadshahi M, 2021, Effect of synbiotic and probiotic supplementation on serum brain-derived neurotrophic factor level, depression and anxiety symptoms in hemodialysis patients: A randomized, double-blinded, clinical trial. *Nutr Neurosci*, 24: 490–499.

https://doi.org/10.1080/1028415X.2019.1646975

123. Marx W, Lane M, Hockey M, *et al.*, 2021, Diet and depression: Exploring the biological mechanisms of action. *Mol Psychiatry*, 26: 134–150.

https://doi.org/10.1038/s41380-020-00925-x

124. Okereke OI, Vyas CM, Mischoulon D, *et al.*, 2021, Effect of long-term supplementation with marine omega-3 fatty acids vs placebo on risk of depression or clinically relevant depressive symptoms and on change in mood scores: A randomized clinical trial. *JAMA*, 326: 2385–2394.

https://doi.org/10.1001/jama.2021.21187

125. Liu RT, Walsh RFL, Sheehan AE, 2019, Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. *Neurosci Biobehav Rev*, 102: 13–23.

https://doi.org/10.1016/j.neubiorev.2019.03.023

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REVIEW ARTICLE

Anti-leucine-rich glioma inactivated-1 autoimmune encephalitis: A review of diagnosis and treatment

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Abstract

Anti-leucine-rich glioma inactivated-1 (LGI1) autoimmune encephalitis is the second most common autoimmune encephalitis, usually with acute or subacute onset. The rates of misdiagnosis and missed diagnosis are high because of its insidious onset. We review the pathogenesis, clinical manifestations, differential diagnosis, treatment, and prognosis of anti-LGI1 autoimmune encephalitis, so as to provide references for clinicians to understand this disease. This disease presents with a variety of clinical manifestations, including faciobrachial dystonic seizure (FBDS), cognitive impairment, hyponatremia, hyperkinetic movements (HMs), and mental impairment. ^{18}F-fluorodeoxyglucose position emission tomography (^{18}F-FDG PET) has higher sensitivity than magnetic resonance imaging (MRI) and can be used to measure disease activity and assess patient response to treatment. The detection of LGI1 antibodies in cerebrospinal fluid or serum is a confirmatory test. The rapid initiation of immunotherapy after diagnosis can significantly improve the prognosis of patients.

Keywords: Autoimmune encephalitis; Leucine-rich glioma-inactivated-1; Faciobrachial dystonic seizure; Immunotherapy

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1. Introduction

Autoimmune encephalitis (AE) generally refers to a type of encephalitis that is mediated by autoimmune mechanisms. AE can be divided into three main types according to different anti-neuronal antibodies and their corresponding clinical syndromes: anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis, limbic encephalitis (including anti-leucine-rich glioma inactivated-1 [LGI1] antibody, anti-γ-aminobutyric acid type B receptor [$GABA_BR$] antibody, and anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor [AMPAR] antibody-related encephalitis), and other AE syndromes[1]. LGI1 is a neuronal secretory glycoprotein, mainly expressed in the hippocampus and temporal lobe cortex. It participates in the development and physiological function of the central nervous system[2]. LGI1 antibody-associated limbic encephalitis was discovered by Irani *et al*. in 2010, accounting for about 25% of autoimmune encephalitis[2]. It is common in

the elderly, affecting more males than females. Its clinical manifestations are characterized by faciobrachial dystonic seizure (FBDS), cognitive disorders, mental disorders, and refractory hyponatremia.

2. Etiology and pathogenesis

LGI1 is a glycoprotein secreted by the presynaptic membrane. It is mainly expressed in the hippocampus and temporal cortex but is also weakly expressed in the basal ganglia and the inner cerebral cortex[3,4]. It connects presynaptic voltage-gated potassium channels (VGKC) and postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR)[5,6]. LGI1 antibody inhibits the binding of LGI1 protein to A Disintegrin And Metalloproteinase 22/23 (ADAM22/23)[7], a metalloproteinase in the synaptic space, through its epitempin (EPTP) domain, and reduces the AMPAR function of inhibitory interneurons. In the hippocampus, inhibitory interneurons constitute a strong feedback or feed forward inhibition loop; thus, the decreased activity of inhibitory interneurons may lead to over-excitation of the whole network[8], resulting in the occurrence of FBDS. It has been found[5] that the knockout of LGI1 gene may cause autosomal dominant lateral temporal epilepsy (ADLTE)[9,10].

Fukata *et al.*[11] showed that the LGI1-ADAM22 complex can form a cross-synaptic heterodimer complex, which is composed of three major parts: the presynaptic part, which includes ADAM23 and the Kv1 channel; the synaptic cleft part, which includes LGI1 protein; and the postsynaptic part, which includes ADAM22, postsynaptic density protein 95 (PSD-95), regulatory protein Stargazin, and AMPAR (Figure 1)[12]. In the physiological state, the Kv1 channel that is located in the presynaptic membrane is activated at the membrane potential near the discharge threshold of neuron[13], which limits and delays the discharge of neuron, thus inhibiting the secretion of the excitatory neurotransmitter glutamate. Glutamate activates AMPAR in the postsynaptic membrane, and the opening of the sodium (Na^+) influx channel mediates postsynaptic excitatory current[14]. Several studies have shown that the loss of LGI1 increases excitatory synaptic transmission, whereas the overexpression of LGI1 decreases glutamate synaptic transmission[15-17]. In a study, Schulte *et al.* transfected oocytes with plasmids expressing Kv1.1, Kv1.4, and Kvβ1 channels and electrophysiologically recorded potassium currents in the presence of endogenous LGI1. They observed that LGI1 prevented the rapid inactivation of Kv1.1 channel via the Kvβ1 subunit. Therefore, it was speculated that LGI1 antibody may mediate the rapid inactivation of Kv1 channel. Lancaster *et al.* have also demonstrated that independent ADAM22 alone had no effect on Kv1.1 current, while the presence of LGI1

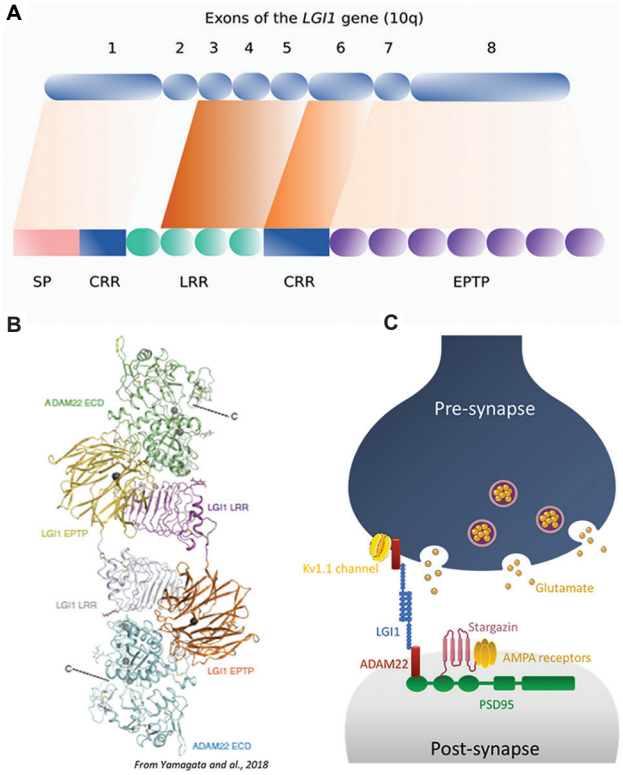


Figure 1. (A-C) *LGI1* gene structure and interaction. (Adapted from Fels *et al.*, 2021, Role of LGI1 protein in synaptic transmission: From physiology to pathology, *Neurobiol Dis*, 160: 105537. http://doi.org/10.1016/j.nbd.2021.105537).

and ADAM22 increased Kv1.1 current[18]. In addition, it has been shown that LGI1 antibodies can induce the internalization of Kv1 channel and its protein complex[19]. These may be the possible mechanisms of FBDS in patients with antibody-LGI1 autoimmune encephalitis (anti-LGI1 AE).

Multiple studies have shown that anti-LGI1 AE is related to human leukocyte antigen (HLA)[20-25], and Ding *et al.* have reported a familial case supporting this genetic association[26]. To date, several studies have conducted HLA genotyping and genome-wide association studies (GWAS) on anti-LGI1 AE patients in different populations, including the Caucasian population[22,23,25,27], Korean population[24], and Southwest Han population[21], revealing significant associations between unique HLA subtypes and anti-LGI1 AE. A genome-wide association study (GWAS) that was conducted in Germany[23] has found that anti-LGI1 AE is associated with 27 single nucleotide polymorphisms (SNPs) that are located in the major histocompatibility complex II (*MHC II*) gene region (between *HLA-DRB1* and *HLA-DQA1*) and that *DRB1*07:01* and *DQA1*02:01* always appeared in all patients. In addition, a study in South Korea has found a high frequency of *B*44:03* and

*C*07:06 HLA I* alleles in patients with anti-LGI1 AE, suggesting that the pathogenesis of this disease may also be related to *HLA I* genes[24]. A French study that performed HLA genotyping in patients with anti-LGI1 AE[27] has found that 88% of patients carried *DRB1*07:01*, with noncarriers being more likely to be young women and less likely to have psychiatric symptoms. It may be that estrogen can alter the expression of HLA[28]. However, the only study in the Chinese Han population has shown no evidence of association between *DRB1*07:01~DQB1*02:02* haplotypes and this disease[21]. These inconsistent results may be due to the fact that the study populations are of different races; thus, it is speculated that genetic factors may play an important role in the etiology of this disease. Future studies with larger samples and more races are needed to verify this hypothesis.

In recent years, several literatures have reported that anti-LGI1 AE may be a rare complication of coronavirus disease (COVID-19) vaccination[29,30], with a strict time relationship between the onset and vaccination (about 2 weeks from vaccination to disease onset). Molecular mimicry and immune cross reaction may be involved in its pathogenesis. Asioli *et al.* have reported four patients who developed anti-LGI1 AE after receiving two different messenger ribonucleic acid (mRNA) vaccines, with one patient vaccinated with a viral vector vaccine. However, since there are too few reported cases of vaccination-related anti-LGI1 AE, it is not possible to determine whether the cause of the disease is related to specific types of COVID-19 vaccines.

3. Clinical manifestations

Most patients have an acute or subacute onset, with the most prominent manifestation being FBDS. Other manifestations include cognitive disorders (mainly recent memory loss), hyponatremia, hyperkinetic movements (HMs), psychiatric disorders, as well as mood and sleep disorders.

3.1. Faciobrachial dystonic seizure (FBDS)

FBDS is highly specific, with a positive rate of approximately 20 – 40%[31,32]. It often occurs as the initial symptom of anti-LGI1 AE[31,33]. FBDS is a frequent and transient involuntary movement like dystonia, which often involves unilateral upper limbs, face, and even lower extremities. Its attacks are usually short, lasting only few seconds, but the frequency of attacks may reach dozens of times a day[1]. Traumatic falls may occur when it manifests as FBDS of the lower limbs. The results of previous studies have indicated that antiepileptic drugs (AEDs) alone are ineffective for FBDS, but the use of immunotherapy can significantly reduce or even eliminate FBDS[32,34-39]. In a study conducted by Thompson *et al.*[31], FBDS ceased in only 10% of anti-LGI1 AE patients who took AEDs alone; in contrast, the cessation of FBDS was observed 30 days after immunotherapy in 51% of patients and even earlier in cognitively normal patients. The current findings on the origin of FBDS and whether it should be regarded as an epileptic seizure are controversial[40]. Some scholars call it a seizure-like episode[41]. In a study, Liu *et al.*[42] divided LGI1 AE patients into two groups, the FBDS group and the non-FBDS group. ^{18}F-fluorodeoxyglucose position emission tomography (^{18}F-FDG-PET) results showed that basal ganglia (BG) hypermetabolism was detected in seven patients (44%) in the FBDS group but in only one patient (7%) in the non-FBDS group. It has been hypothesized that BG abnormalities may be involved in the etiology of FBDS and LGI1-associated FBDS is more likely a dyskinesia rather than an epileptic disease. However, in another study by Liu *et al.*[43], anti-LGI1 AE patients initially presented with FBDS, followed by classic limbic epilepsy or ipsilateral tonic-clonic seizures (TCS), and electroencephalogram (EEG) recorded changes in cortical potentials. These specific, stereotyped clinical presentations and EEG changes reflected both cortical and subcortical network involvement rather than a single site. They therefore speculated that FBDS is more likely to be a part of epileptic motor events.

3.2. Cognitive disorders

The majority of patients have rapidly progressive memory impairment, short-term memory loss, and impaired orientation. Other clinical manifestations include language ability deficits, impaired executive function, impaired visuospatial ability, and calculation disturbance[44]. Brain magnetic resonance imaging (MRI) examination shows that the hippocampus, temporal lobe, and basal ganglia are the main lesion sites[37,45,46-49]. At present, it is believed that inflammation in the acute phase is associated with atrophy in the recovery stage, which may be the cause of persistent cognitive impairment in patients with anti-LGI1 AE. Studies by Thompson *et al.*[31] have shown that patients with both cognitive impairment and FBDS have more abnormal examination results than patients with FBDS alone. Abnormal findings of brain MRI and electroencephalogram and hyponatremia were almost exclusively observed in patients with cognitive impairment. There is evidence that cognitive impairment can be prevented by early termination of FBDS[31].

3.3. Hyponatremia

Hyponatremia is another prominent feature of anti-LGI1 AE. As shown in the previous studies, 60% of patients developed hyponatremia after early treatment of the

disease[50]. The pathogenic mechanism of hyponatremia is likely associated with the syndrome of inappropriate antidiuretic hormone secretion caused by the simultaneous expression of LGI1 by the hypothalamus and the kidney[51]. Sometimes, severe hyponatremia can be seen as a precursor to anti-LGI1 AE. The serum sodium concentration of patients with refractory hyponatremia who have been receiving continuous intravenous and oral sodium supplementation can no longer be maintained at the normal level. As a result, they need to take concentrated sodium for a long time and recheck their serum sodium concentration regularly after being discharged from the hospital.

3.4. Hyperkinetic movements (HMs)

In contrast to the extensive discussion on FBDS, HMs have been overlooked in the past. HMs are characterized as a group of jerk-like or twisting movements[43]. This type of manifestation has only been described in a few case reports[43,52-54]. HMs may occur when the patient is asleep or awake, and they do not interrupt the patient's autonomous movements. As a result, only a small number of patients would notice such symptoms and inform the doctor at the time of admission. According to Liu *et al.*[43], HMs are very common in anti-LGI1 AE and can be promoted by sleep. Therefore, it is inferred that HMs are the result of the imbalance of the motor cortex, basal ganglia, thalamus, and substantia nigra/red nucleus. HMs may also be the potential cause of sleep disorders.

3.5. Psychiatric disorders

Results from the previous studies have shown that the incidence of psychiatric symptoms in patients with anti-LGI1 AE is significant. The main manifestations are personality and behavioral disorders, irritability, anxiety, impulsive behavior, hallucinations, delusions, and coma[50,51]. In a South Korean study by Jang *et al.*[55], 62.5% of patients with anti-LGI1 AE developed psychiatric symptoms that were associated with mood, with depressed mood being the most common symptom, while others such as irritability, self-injurious behavior, and emotional instability were also observed. These mood-related symptoms may mislead patients and their clinicians into considering the illness as a psychiatric disorder, such as geriatric depression, early behavioral and psychological symptoms of dementia (BPSD), or epilepsy-related psychosis. As a result, a considerable number of patients may visit the psychiatric department first, thus precluding the early diagnosis of anti-LGI1 AE. Among the various sleep disorders, insomnia is the most common. Insomnia is characterized by difficulty in falling asleep or lack of sleep caused by frequent waking. These symptoms are mainly related to hippocampal and amygdala impairment[56]. These symptoms can reflect the severity of the disease and the effectiveness of treatment to a certain extent.

4. Supplementary examination

4.1. Cerebrospinal fluid examination

Cerebrospinal fluid examinations of patients with anti-LGI1 AE are often non-inflammatory and without any apparent specificity. Lumbar puncture pressure can be normal or slightly increased, and the white blood cell count in cerebrospinal fluid can also be normal or slightly increased, especially lymphocytes[1,51,57]. Cerebrospinal fluid routine biochemical examinations are usually normal. However, few patients may show slightly increased cerebrospinal fluid protein and glucose[58], with positive oligoclonal bands[59].

4.2. Brain magnetic resonance imaging (MRI)

Brain MRI is the first choice for an early diagnosis of anti-LGI1 AE. In most patients, brain MRI shows unilateral or bilateral medial temporal lobes (amygdala and hippocampus) with long T1 and long T2 signals in the acute phase, and slightly higher signals can be seen on diffusion-weighted imaging (DWI) sequence. T2-fluid-attenuated inversion recovery (FLAIR) hyperintensity can be seen on both plain and enhanced MRI[58]. Amygdaloid hypertrophy, which is sensitive to FLAIR sequence, can be found in some patients. Abnormal signals in basal ganglia can also be observed in the brain MRIs of some patients. However, the brain MRIs of some patients may be normal. Significant reductions in brain connectivity involving inferior frontal gyrus, anterior and posterior cingulate gyri, several regions of the default mode network (DMN), and higher visual networks have been demonstrated in patients with anti-LGI1 AE using resting-state functional MRI (fMRI)[3]. Irani *et al.*[38] have shown that the hippocampal volume of patients was significantly smaller than that of matched normal controls during the follow-up period after recovery, although the brain MRIs revealed no abnormal signals in their hippocampus in the acute phase, thus suggesting that anti-LGI1 AE may be caused by brain atrophy.

4.3. ^{18}F-fluorodeoxyglucose position emission tomography (^{18}F-FDG PET)

According to current research, ^{18}F-FDG-PET is considered to be more sensitive than MRI in the diagnosis of anti-LGI1 AE[42,60]. Parietal and frontal cortex hypometabolism and medial temporal lobe (MTL), basal ganglia (BG), and occipital lobe hypermetabolism can be detected by ^{18}F-FDG PET in patients with anti-LGI1 AE[42]. The hypometabolism may be a result of functional impairment propagated along cortical and subcortical networks, arising from the sites of primary abnormalities in MTL and BG[3]. Occipital

hypermetabolism, on the other hand, may be a compensatory mechanism during the acute/early phase of the illness. In the acute phase of the disease, FDG uptake shows a significant increase, followed by a gradual decrease, which eventually returns to normal after treatment[46,61]. Therefore, ^{18}F-FDG PET can be used as a tool to measure disease activity and evaluate patients' responses to treatment.

4.4. Electroencephalogram (EEG)

The proportion of abnormal EEG during FBDS attacks is only 21% to 30%. During the interictal period of FBDS, mild diffuse slow wave or bilateral frontotemporal slow wave can be seen, or the EEG may also be completely normal[37]. Navarro *et al*. have found that FBDS originates from the primary motor cortex (M1)[35]. Before muscle contraction, EEG shows focal, contralateral, and frontal slow waves. The previous studies have demonstrated the superiority of long-lasting EEG monitoring over short-lasting routine EEG in revealing subclinical and recurrent seizures[62,63], which enables clinicians to adjust the treatment plan in time.

4.5. Antibody detection

Anti-LGI1 antibodies in serum and/or cerebrospinal fluid are positive. It is worth noting that the detection of LGI1 antibodies in the serum seems to be more sensitive than that in cerebrospinal fluid compared to other types of autoimmune encephalitis[20,32,54]. Therefore, it is important to detect both types of samples when anti-LGI1 AE is clinically suspected[27]. According to Zhong *et al.*[64], the concentration of LGI1 antibody in the cerebrospinal fluid is related to the probability of recurrence of anti-LGI1 AE, in which the higher the concentration, the greater the probability of recurrence.

4.6. Tumor markers

According to the previous studies, the incidence of tumors in anti-LGI1 AE patients was found to be 0% to 13%[43], which was comparatively less than other types of autoimmune encephalitis[65,66]. In addition, the serum tumor markers of anti-LGI1 AE patients are often negative. Due to the lack of specific recommendations for tumor screening in anti-LGI1 AE, the tumor screening methods for paraneoplastic syndromes (PNS)[67] can be referenced: a second screening shall be repeated after 3–6 months, followed by regular screening every 6 months for 4 years. Patients with concurrent neoplasia may seek chemotherapy, radiotherapy, and/or surgery after AE is controlled.

5. Differential diagnosis

Anti-LGI1 AE needs to be differentiated from central nervous system infection (such as herpes simplex virus meningitis), metabolic encephalopathy, toxic encephalopathy (such as Wernicke's encephalopathy, hepatic encephalopathy, and pulmonary encephalopathy), Hashimoto's encephalopathy[68], central nervous system neoplasms (such as cerebral gliomatosis, primary central nervous system lymphoma, and metastatic cancer), hereditary diseases (such as mitochondrial encephalopathy, methylmalonic acidemia, and adrenoleukodystrophy), and neurodegenerative diseases (such as Creutzfeldt-Jakob disease, Lewy body dementia, multiple system atrophy, and hereditary cerebellar degeneration).

6. Treatment and prognosis

Early identification, diagnosis, and rapid initiation of immunotherapy are all very important to improve the prognosis of patients with anti-LGI1 AE[31,38]. However, the median time from onset to diagnosis of patients with anti-LGI1 AE in China is about 2 months[69], indicating that many patients would have had missed the best opportunity for diagnosis and treatment.

First-line immunotherapy, including glucocorticoids, intravenous immunoglobulin (IVIg), and plasma exchange, is the preferred treatment. The previous studies have shown that immunotherapy is particularly effective in achieving control of FBDS and other seizures. Patients who receive glucocorticoids (intravenous, oral, or both) tend to have better short-term prognosis than those receiving IVIg alone. This may be due to the fact that LGI1 antibodies mainly belong to the IgG4 subclass, which cannot activate complements; thus, steroids have a stronger immunosuppressive effect on these antibodies, while IVIg has a lower efficacy in these diseases[70,71]. The side effects of glucocorticoid therapy are common, especially those affecting the musculoskeletal system (myopathy, tendon rupture, and osteoporosis). Other rare but serious complications include acute respiratory distress syndrome (ARDS), septicemia, and pulmonary embolism (PE)[31]. Zhang *et al.*[72] have demonstrated that plasmapheresis can promptly relieve the symptoms of patients without fatal adverse events. However, it is more suitable for the emergency treatment of critically ill patients so as to achieve faster remission in view of its high treatment cost and less trauma.

Second-line therapeutic agents include rituximab and IV cyclophosphamide[1]. They are mainly used for patients with poor effect of first-line immunotherapy (Figure 2)[73], which account for 10% to 15% of all anti-LGI1 AE patients. Rituximab can be used when the patient's condition has not significantly improved or is aggravated after 2 weeks of treatment with more than two kinds of first-line immunotherapies. Cyclophosphamide may be used when rituximab is not available or contraindicated. Rituximab is

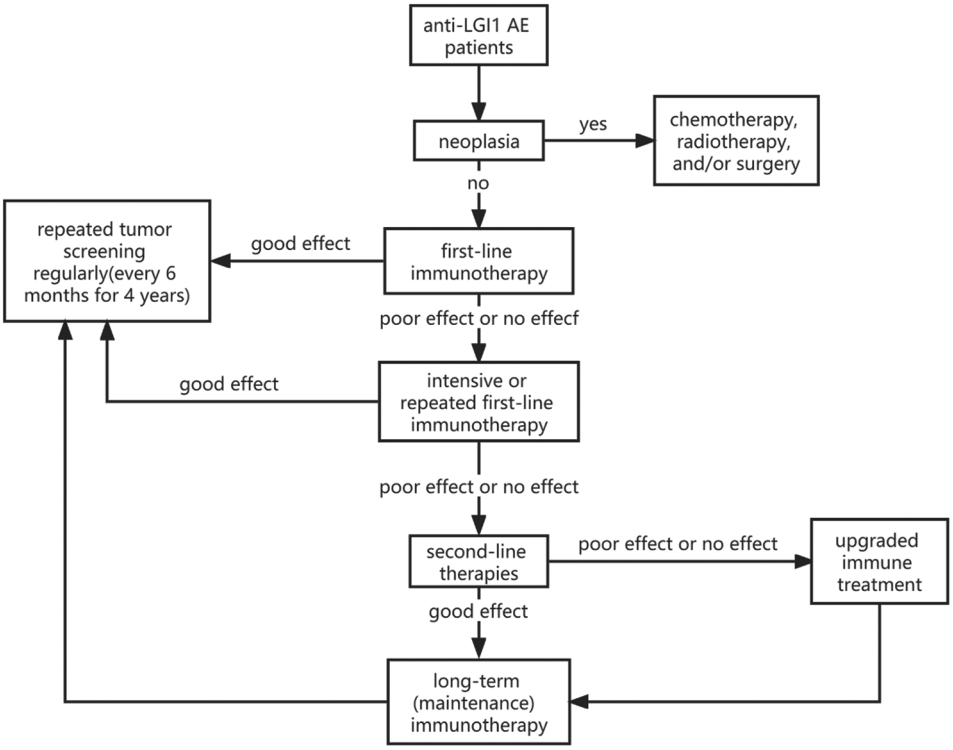


Figure 2. Immunotherapy flow chart for anti-leucine-rich glioma inactivated-1 (LGI1) autoimmune encephalitis.

prescribed at 375 mg/m^2 (up to 1 g) once weekly for four consecutive weeks[1]. It has been found that rituximab is effective in anti-LGI1 AE patients and well-tolerated in elderly patients[74,75]. Common adverse reactions of rituximab include infusion-related reactions (IRRS), pneumonia, severe sepsis, and so on[44].

The overall effect of antiepileptic drugs (AEDs) is limited, and immunotherapy is more effective in controlling FBDS and seizures, so AEDs are only used as an add-on therapy to immunotherapy. Immunotherapy is viewed as an etiological treatment, whereas antiepileptic therapy is a symptomatic approach. A nationwide cohort study from the Netherlands has suggested that sodium channel blockers (carbamazepine, oxcarbazepine, *etc.*) may be more effective in patients with anti-LGI1 AE[76]. The study, which included 53 patients, showed that carbamazepine was more effective than levetiracetam in reducing seizures. Compared to patients with other types of AE, those with anti-LGI1 AE have been reported to have a higher incidence of adverse reactions to AEDs. Marienke *et al.* have reported in their study that one-third of patients who were prescribed with carbamazepine developed rash. A possible reason for that was the rapid increase in the dose of carbamazepine due to frequent drug-resistant seizures. Therefore, caution must be exercised when increasing the dose of AEDs. IV bolus diazepam or intramuscular midazolam can be administered to terminate status epilepticus (SE).

At present, there are very few studies on the effect of drug therapy on cognitive impairment. An early initiation of immunotherapy will not only help control the onset of FBDS, but also improve the cognitive function of patients. Both donepezil and memantine are classic cognitive-improving agents for Alzheimer's disease, but there is no literature to report the efficacy of these agents in patients with anti-LGI1 AE.

A recent large cohort study of the recurrence rate of anti-LGI1 AE in western China population[77] has shown that the recurrence rate of anti-LGI1 AE patients is 13.6%. According to another study in northeast China[64], the recurrence rate is 28.6%. The recurrence rate varies from study to study, indicating that there is heterogeneity in anti-LGI1 AE in different ethnic populations. In addition, considering that relapses may occur in several years after the initial onset, the recurrence rate may be underestimated. Inadequate dose and duration of the drug may be the cause of recurrence[32]. Some scholars believe that acute sleep disorders are risk factors for the recurrence of anti-LGI1 AE[77]. In a study using ^{18}F-FDG-PET, the recurrence rate of patients with high metabolism of BG alone or both BG and MTL was lower than that of patients with high metabolism of MTL alone[42]. Overall, a consensus on the factors affecting recurrence has not been achieved; hence, more long-term follow-up

studies are required to further determine the causes of recurrence.

A mortality rate of 6% has been reported in patients with anti-LGl1 AE[78]. Persistent cognitive impairment is a long-term sequela of patients with anti-LGl1 AE, especially in the absence of early and appropriate immunotherapy. This irreversible cognitive impairment in patients may be due to high levels of LGI1 antibody in their cerebrospinal fluid, followed by complement deposition and hippocampal atrophy[49,78-80]. Thompson *et al.*[31,78] have emphasized the importance of early and effective immunotherapy for patients. For each week of delay in immunotherapy, the possibility of FBDS resolution decreases by 5%. If FBDS persists for more than 90 days, more than half of the patients will develop cognitive impairment. The time needed to recover from cognitive impairment is associated with the time immunotherapy is initiated. Early initiation of immunotherapy may reduce FBDS and, more likely, prevent long-term cognitive impairment.

In addition, the prognosis of a patient is largely affected by the occurrence of tumor. A study in the Netherlands has found that non-neoplastic anti-LGI1 AE is strongly associated with *HLA-DR7* and *HLA-DRB4*, suggesting that *HLA-DR7* or *HLA-DRB4* gene deletion may increase the incidence of tumors[25]. Therefore, the researchers have recommended intensive tumor screening and long-term follow-up in patients without *HLA-DR7* or *HLA-DRB4*. In contrast, a British study has shown that HLA is not associated with tumors in anti-LGI1 AE[22]. Considering the incongruity, there is an urgent need for more research on HLA and tumors of these patients to guide clinical diagnosis and treatment.

7. Conclusions

Anti-LGI1 AE is a rare condition, and because it often occurs with recent memory loss or mental and behavioral disorders, diagnosis and treatment are often delayed, thus increasing the risk of poor prognosis. The diagnosis of anti-LGI1 AE mainly depends on the history, clinical manifestations, positive results of cerebrospinal fluid or serum LGI1 antibody spectrum tests, and characteristic brain MRI and ^{18}F-FDG PET results. Patients with anti-LGI1 AE require early diagnosis, while missed diagnosis or misdiagnosis should be prevented. An early initiation of immunotherapy can effectively reduce mortality, seizures, and the incidence of long-term sequelae; it can also prevent borderline encephalitis and long-term cognitive impairment. More prospective studies are needed in the future since the majority of existing studies are retrospective studies. The review of relevant literature may help improve clinicians' understanding of the characteristic manifestations of the disease.

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Conflict of interest

The authors report no conflict of interest.

Author contributions

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References

1. Wang JD, Xie L, Fang X, *et al.*, 2022, Clinical validation of the 2020 diagnostic approach for pediatric autoimmune encephalitis in a single center. *Zhonghua Er Ke Za Zhi*, 60: 786–791.

   https://doi.org/10.3760/cma.j.cn112140-20220111-00039

2. Baudin P, Cousyn L, Navarro V, *et al.*, 2021, The LGI1 protein: Molecular structure, physiological functions and disruption-related seizures. *Cell Mol Life Sci*, 79: 16.

   https://doi.org/10.1007/s00018-021-04088-y

3. Heine J, Pruss H, Kopp UA, *et al.*, 2018, Beyond the limbic system: Disruption and functional compensation of large-scale brain networks in patients with anti-LGI1 encephalitis. *J Neurol Neurosurg Psychiatry*, 89: 1191–1199.

   https://doi.org/10.1136/jnnp-2017-317780

4. Herranz-Perez V, Olucha-Bordonau FE, Morante-Redolat JM, *et al.*, 2010, Regional distribution of the leucine-rich glioma inactivated (LGI) gene family transcripts in the adult mouse brain. *Brain Res*, 1307: 177–194.

   https://doi.org/10.1016/j.brainres.2009.10.013

5. Ohkawa T, Fukata Y, Yamasaki M, *et al.*, 2013, Autoantibodies to epilepsy-related LGI1 in limbic encephalitis neutralize LGI1-ADAM22 interaction and reduce synaptic AMPA receptors. *J Neurosci*, 33: 18161–18174.

https://doi.org/10.1523/JNEUROSCI.3506-13.2013

6. Fukata Y, Lovero KL, Iwanaga T, *et al*., 2010, Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. *Proc Natl Acad Sci U S A*, 107: 3799–3804.

https://doi.org/10.1073/pnas.0914537107

7. Petit-Pedrol M, Sell J, Planaguma J, *et al*., 2018, LGI1 antibodies alter Kv1.1 and AMPA receptors changing synaptic excitability, plasticity and memory. *Brain*, 141: 3144–3159.

https://doi.org/10.1093/brain/awy253

8. Acsady L, Kamondi A, Sik A, *et al*., 1998, GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J Neurosci*, 18: 3386–3403.

9. Kalachikov S, Evgrafov O, Ross B, *et al*., 2002, Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nat Genet*, 30: 335–341.

https://doi.org/10.1038/ng832

10. Morante-Redolat JM, Gorostidi-Pagola A, Piquer-Sirerol S, *et al*., 2002, Mutations in the LGI1/epitempin gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Hum Mol Genet*, 11: 1119–1128.

https://doi.org/10.1093/hmg/11.9.1119

11. Yuko F, Xiumin C, Satomi C, *et al*., 2021, LGI1-ADAM22-MAGUK configures transsynaptic nanoalignment for synaptic transmission and epilepsy prevention. *Proc Natl Acad Sci U S A*, 118: e2022580118.

https://doi.org/10.1073/pnas.2022580118

12. Fels E, Muniz-Castrillo S, Vogrig A, *et al*., 2021, Role of LGI1 protein in synaptic transmission: From physiology to pathology. *Neurobiol Dis*, 160: 105537.

https://doi.org/10.1016/j.nbd.2021.105537

13. Guan D, Lee JC, Tkatch T, *et al*., 2006, Expression and biophysical properties of Kv1 channels in supragranular neocortical pyramidal neurones. *J Physiol*, 571(Pt 2): 371–389.

https://doi.org/10.1113/jphysiol.2005.097006

14. Henley JM, Nair JD, Seager R, *et al*., 2021, Kainate and AMPA receptors in epilepsy: Cell biology, signalling pathways and possible crosstalk. *Neuropharmacology*, 195: 108569.

https://doi.org/10.1016/j.neuropharm.2021.108569

15. Boillot M, Lee CY, Allene C, *et al*., 2016, LGI1 acts presynaptically to regulate excitatory synaptic transmission during early postnatal development. *Sci Rep*, 6: 21769.

https://doi.org/10.1038/srep21769

16. Zhou YD, Lee S, Jin Z, *et al*., 2009, Arrested maturation of excitatory synapses in autosomal dominant lateral temporal lobe epilepsy. *Nat Med*, 15: 1208–1214.

https://doi.org/10.1038/nm.2019

17. Fukata Y, Adesnik H, Iwanaga T, *et al*., 2006, Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. *Science*, 313: 1792–1795.

https://doi.org/10.1126/science.1129947

18. Lancaster E, Burnor E, Zhang J, *et al*., 2019, ADAM23 is a negative regulator of Kv1.1/Kv1.4 potassium currents. *Neurosci Lett*, 704: 159–163.

https://doi.org/10.1016/j.neulet.2019.04.012

19. Ramberger M, Berretta A, Tan JM, *et al*., 2020, Distinctive binding properties of human monoclonal LGI1 autoantibodies determine pathogenic mechanisms. *Brain*, 143: 1731–1745.

https://doi.org/10.1093/brain/awaa104

20. Muniz-Castrillo S, Vogrig A, Honnorat J, *et al*., 2020, Associations between HLA and autoimmune neurological diseases with autoantibodies. *Auto Immun Highlights*, 11: 2.

https://doi.org/10.1186/s13317-019-0124-6

21. Hu F, Liu X, Zhang L, *et al*., 2020, Novel findings of HLA association with anti-LGI1 encephalitis: HLA-DRB1*03:01 and HLA-DQB1*02:01. *J Neuroimmunol*, 344: 577243.

https://doi.org/10.1016/j.jneuroim.2020.577243

22. Binks S, Varley J, Lee W, *et al*., 2018, Distinct HLA associations of LGI1 and CASPR2-antibody diseases. *Brain*, 141: 2263–2271.

https://doi.org/10.1093/brain/awy109

23. Mueller SH, Farber A, Pruss H, *et al*., 2018, Genetic predisposition in anti-LGI1 and anti-NMDA receptor encephalitis. *Ann Neurol*, 83: 863–869.

https://doi.org/10.1002/ana.25216

24. Kim TJ, Lee ST, Moon J, *et al*., 2017, Anti-LGI1 encephalitis is associated with unique HLA subtypes. *Ann Neurol*, 81: 183–192.

https://doi.org/10.1002/ana.24860

25. Van Sonderen A, Roelen DL, Stoop JA, *et al*., 2017, Anti-LGI1 encephalitis is strongly associated with HLA-DR7 and HLA-DRB4. *Ann Neurol*, 81: 193–198.

https://doi.org/10.1002/ana.24858

26. Ding C, Sun Q, Li R, *et al*., 2022, The first case of familiar anti-leucine-rich glioma-inactivated 1 autoimmune encephalitis: A case report and literature review. *Front Neurol*, 13: 855383.

https://doi.org/10.3389/fneur.2022.855383

27. Muniz-Castrillo S, Haesebaert J, Thomas L, *et al*., 2021, Clinical and prognostic value of immunogenetic characteristics in anti-LGI1 encephalitis. *Neurol Neuroimmunol Neuroinflamm*, 8: e974.

https://doi.org/10.1212/NXI.0000000000000974

28. Taneja V, 2018, Sex hormones determine immune response. *Front Immunol*, 9: 1931.

https://doi.org/10.3389/fimmu.2018.01931

29. Asioli GM, Muccioli L, Barone V, *et al*., 2022, Anti-LGI1 encephalitis following COVID-19 vaccination: A case series. *J Neurol*, 269: 5720–5723.

https://doi.org/10.1007/s00415-022-11234-4

30. Zlotnik Y, Gadoth A, Abu-Salameh I, *et al*., 2021, Case report: Anti-LGI1 encephalitis following COVID-19 vaccination. *Front Immunol*, 12: 813487.

https://doi.org/10.3389/fimmu.2021.813487

31. Thompson J, Bi M, Murchison AG, *et al*., 2018, The importance of early immunotherapy in patients with faciobrachial dystonic seizures. *Brain*, 141: 348–356.

https://doi.org/10.1093/brain/awx323

32. Van Sonderen A, Thijs RD, Coenders EC, *et al*., 2016, Anti-LGI1 encephalitis: Clinical syndrome and long-term follow-up. *Neurology*, 87: 1449–1456.

https://doi.org/10.1212/WNL.0000000000003173

33. Simabukuro MM, Nobrega PR, Pitombeira M, *et al*., 2016, The importance of recognizing faciobrachial dystonic seizures in rapidly progressive dementias. *Dement Neuropsychol*, 10: 351–357.

https://doi.org/10.1590/s1980-5764-2016dn1004016

34. Gastaldi M, Thouin A, Vincent A, *et al*., 2016, Antibody-mediated autoimmune encephalopathies and immunotherapies. *Neurotherapeutics*, 13: 147–162.

https://doi.org/10.1007/s13311-015-0410-6

35. Navarro V, Kas A, Apartis E, *et al*., 2016, Motor cortex and hippocampus are the two main cortical targets in LGI1-antibody encephalitis. *Brain*, 139(Pt 4): 1079–1093.

https://doi.org/10.1093/brain/aww012

36. Irani SR, Buckley C, Vincent A, *et al*., 2008, Immunotherapy-responsive seizure-like episodes with potassium channel antibodies. *Neurology*, 71: 1647–1648.

https://doi.org/10.1212/01.wnl.0000326572.93762.51

37. Irani SR, Michell AW, Lang B, *et al*., 2011, Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Ann Neurol*, 69: 892–900.

https://doi.org/10.1002/ana.22307

38. Irani SR, Stagg CJ, Schott JM, *et al*., 2013, Faciobrachial dystonic seizures: The influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain*, 136(Pt 10): 3151–3162.

https://doi.org/10.1093/brain/awt212

39. Flanagan EP, Kotsenas AL, Britton JW, *et al*., 2015, Basal ganglia T1 hyperintensity in LGI1-autoantibody faciobrachial dystonic seizures. *Neurol Neuroimmunol Neuroinflamm*, 2: e161.

https://doi.org/10.1212/NXI.0000000000000161

40. Striano P, 2011, Faciobrachial dystonic attacks: Seizures or movement disorder? *Ann Neurol*, 70: 179–180; author reply 180.

https://doi.org/10.1002/ana.22470

41. Barajas RF, Collins DE, Cha S, *et al*., 2010, Adult-onset drug-refractory seizure disorder associated with anti-voltage-gated potassium-channel antibody. *Epilepsia*, 51: 473–477.

https://doi.org/10.1111/j.1528-1167.2009.02287.x

42. Liu X, Shan W, Zhao X, *et al*., 2020, The clinical value of (18) F-FDG-PET in autoimmune encephalitis associated with LGI1 antibody. *Front Neurol*, 11: 418.

https://doi.org/10.3389/fneur.2020.00418

43. Liu X, Han Y, Yang L, *et al*., 2020, The exploration of the spectrum of motor manifestations of anti-LGI1 encephalitis beyond FBDS. *Seizure*, 76: 22–27.

https://doi.org/10.1016/j.seizure.2019.12.023

44. Teng Y, Li T, Yang Z, *et al*., 2021, Clinical features and therapeutic effects of anti-leucine-rich glioma inactivated 1 encephalitis: A systematic review. *Front Neurol*, 12: 791014.

https://doi.org/10.3389/fneur.2021.791014

45. Fauser S, Talazko J, Wagner K, *et al*., 2005, FDG-PET and MRI in potassium channel antibody-associated non-paraneoplastic limbic encephalitis: Correlation with clinical course and neuropsychology. *Acta Neurol Scand*, 111: 338–343.

https://doi.org/10.1111/j.1600-0404.2005.00406.x

46. Lv RJ, Sun ZR, Cui T, *et al*., 2014, Temporal lobe epilepsy with amygdala enlargement: A subtype of temporal lobe epilepsy. *BMC Neurol*, 14: 194.

https://doi.org/10.1186/s12883-014-0194-z

47. Miller TD, Chong TT, Davies AM, *et al*., 2017, Focal CA3 hippocampal subfield atrophy following LGI1 VGKC-complex antibody limbic encephalitis. *Brain*, 140: 1212–1219.

https://doi.org/10.1093/brain/awx070

48. Szots M, Blaabjerg M, Orsi G, *et al*., 2017, Global brain atrophy and metabolic dysfunction in LGI1 encephalitis: A prospective multimodal MRI study. *J Neurol Sci*, 376: 159–165.

https://doi.org/10.1016/j.jns.2017.03.020

49. Finke C, Pruss H, Heine J, *et al*., 2017, Evaluation of cognitive deficits and structural hippocampal damage in encephalitis with leucine-rich, glioma-inactivated 1 antibodies. *JAMA Neurol*, 74: 50–59.

https://doi.org/10.1001/jamaneurol.2016.4226

50. Bien CG, Elger CE, 2007, Limbic encephalitis: A cause of temporal lobe epilepsy with onset in adult life. *Epilepsy*

Behav, 10: 529–538.

https://doi.org/10.1016/j.yebeh.2007.03.011

51. Lai M, Huijbers MG, Lancaster E, *et al*., 2010, Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: A case series. *Lancet Neurol*, 9(8): 776–785.

https://doi.org/10.1016/S1474-4422(10)70137-X

52. Tofaris GK, Irani SR, Cheeran BJ, *et al*., 2012, Immunotherapy-responsive chorea as the presenting feature of LGI1-antibody encephalitis. *Neurology*, 79: 195–196.

https://doi.org/10.1212/WNL.0b013e31825f0522

53. Balint B, Vincent A, Meinck HM, *et al*., 2018, Movement disorders with neuronal antibodies: Syndromic approach, genetic parallels and pathophysiology. *Brain*, 141: 13–36.

https://doi.org/10.1093/brain/awx189

54. Gadoth A, Pittock SJ, Dubey D, *et al*., 2017, Expanded phenotypes and outcomes among 256 LGI1/CASPR2-IgG-positive patients. *Ann Neurol*, 82: 79–92.

https://doi.org/10.1002/ana.24979

55. Jang Y, Lee ST, Lim JA, *et al*., 2018, Psychiatric symptoms delay the diagnosis of anti-LGI1 encephalitis. *J Neuroimmunol*, 317: 08–14.

https://doi.org/10.1016/j.jneuroim.2018.02.005

56. Peter-Derex L, Devic P, Rogemond V, *et al*., 2012, Full recovery of agrypnia associated with anti-Lgi1 antibodies encephalitis under immunomodulatory treatment: A case report with sequential polysomnographic assessment. *Sleep Med*, 13: 554–556.

https://doi.org/10.1016/j.sleep.2012.01.002

57. Jarius S, Hoffmann L, Clover L, *et al*., 2008, CSF findings in patients with voltage gated potassium channel antibody associated limbic encephalitis. *J Neurol Sci*, 268: 74–77.

https://doi.org/10.1016/j.jns.2007.11.004

58. Li Y, Song F, Liu W, *et al*., 2021, Clinical features of nine cases of leucine-rich glioma inactivated 1 protein antibody-associated encephalitis. *Acta Neurol Belg*, 121: 889–897.

https://doi.org/10.1007/s13760-020-01336-z

59. Wang M, Cao X, Liu Q, *et al*., 2017, Clinical features of limbic encephalitis with LGI1 antibody. *Neuropsychiatr Dis Treat*, 13: 1589–1596.

https://doi.org/10.2147/NDT.S136723

60. Rissanen E, Carter K, Cicero S, *et al*., 2022, Cortical and subcortical dysmetabolism are dynamic markers of clinical disability and course in anti-LGI1 encephalitis. *Neurol Neuroimmunol Neuroinflamm*, 9: e1136.

https://doi.org/10.1212/NXI.0000000000001136

61. Tripathi M, Tripathi M, Roy SG, *et al*., 2018, Metabolic topography of autoimmune non-paraneoplastic encephalitis. *Neuroradiology*, 60: 189–198.

https://doi.org/10.1007/s00234-017-1956-2

62. Morano A, Fanella M, Irelli EC, *et al*., 2020, Seizures in autoimmune encephalitis: Findings from an EEG pooled analysis. *Seizure*, 83: 160–168.

https://doi.org/10.1016/j.seizure.2020.10.019

63. Baumgartner T, Pitsch J, Olaciregui-Dague K, *et al*., 2022, Seizure underreporting in LGI1 and CASPR2 antibody encephalitis. *Epilepsia*, 63: e100–e105.

https://doi.org/10.1111/epi.17338

64. Zhong R, Chen Q, Zhang X, *et al*., 2022, Relapses of anti-NMDAR, anti-GABABR and anti-LGI1 encephalitis: A retrospective cohort study. *Front Immunol*, 13: 918396.

https://doi.org/10.3389/fimmu.2022.918396

65. Irani SR, Pettingill P, Kleopa KA, *et al*., 2012, Morvan syndrome: Clinical and serological observations in 29 cases. *Ann Neurol*, 72: 241–255.

https://doi.org/10.1002/ana.23577

66. Irani SR, Alexander S, Waters P, *et al*., 2010, Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain*, 133: 2734–2748.

https://doi.org/10.1093/brain/awq213

67. Titulaer MJ, Soffietti R, Dalmau J, *et al*., 2011, Screening for tumours in paraneoplastic syndromes: Report of an EFNS task force. *Eur J Neurol*, 18: 19–e3.

https://doi.org/10.1111/j.1468-1331.2010.03220.x

68. Maki Y, Takashima H, 2016, Clinical features and treatment of hashimoto encephalopathy. *Brain Nerve*, 68: 1025–1033.

https://doi.org/10.11477/mf.1416200549

69. Huang X., Fan C, Gao L, *et al*., 2022, Clinical features, immunotherapy, and outcomes of anti-leucine-rich glioma-inactivated-1 encephalitis. *J Neuropsychiatry Clin Neurosci*, 34: 141–148.

https://doi.org/10.1176/appi.neuropsych.20120303

70. Galeotti C, Kaveri SV, Bayry J, *et al*., 2017, IVIG-mediated effector functions in autoimmune and inflammatory diseases. *Int Immunol*, 29: 491–498.

https://doi.org/10.1093/intimm/dxx039

71. Rodriguez A, Klein CJ, Sechi E, *et al*., 2022, LGI1 antibody encephalitis: Acute treatment comparisons and outcome. *J Neurol Neurosurg Psychiatry*, 93: 309–315.

https://doi.org/10.1136/jnnp-2021-327302

72. Zhang Y, Huang HJ, Chen WB, *et al*., 2021, Clinical efficacy of plasma exchange in patients with autoimmune

encephalitis. *Ann Clin Transl Neurol*, 8: 763–773.

https://doi.org/10.1002/acn3.51313

73. Ghimire P, Khanal UP, Gajurel BP, *et al.*, 2020, Anti-LGI1, anti-GABABR, and anti-CASPR2 encephalitides in Asia: A systematic review. *Brain Behav*, 10: e01793.

https://doi.org/10.1002/brb3.1793

74. Nepal G, Shing YK, Yadav JK, *et al.*, 2020, Efficacy and safety of rituximab in autoimmune encephalitis: A meta-analysis. *Acta Neurol Scand*, 142: 449–459.

https://doi.org/10.1111/ane.13291

75. Lee WJ, Lee ST, Moon J, *et al.*, 2016, Tocilizumab in autoimmune encephalitis refractory to rituximab: An institutional cohort study. *Neurotherapeutics*, 13: 824–832.

https://doi.org/10.1007/s13311-016-0442-6

76. De Bruijn MA, van Sonderen A, van Coevorden-Hameete MH, *et al.*, 2019, Evaluation of seizure treatment in anti-LGI1, anti-NMDAR, and anti-GABA(B)R encephalitis. *Neurology*, 92: e2185–e2196.

https://doi.org/10.1212/WNL.0000000000007475

77. Guo K, Liu X, Lin J, *et al.*, 2022, Clinical characteristics, long-term functional outcomes and relapse of anti-LGI1/Caspr2 encephalitis: A prospective cohort study in Western China. *Ther Adv Neurol Disord*, 15.

https://doi.org/10.1177/17562864211073203

78. Arino H, Armangue T, Petit-Pedrol M, *et al.*, 2016, Anti-LGI1-associated cognitive impairment: Presentation and long-term outcome. *Neurology*, 87: 759–765.

https://doi.org/10.1212/WNL.0000000000003009

79. Bien CG, Urbach H, Schramm J, *et al.*, 2007, Limbic encephalitis as a precipitating event in adult-onset temporal lobe epilepsy. *Neurology*, 69: 1236–1244.

https://doi.org/10.1212/01.wnl.0000276946.08412.ef

80. Bien CG, Vincent A, Barnett MH, *et al.*, 2012, Immunopathology of autoantibody-associated encephalitides: Clues for pathogenesis. *Brain*, 135(Pt 5): 1622–1638.

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REVIEW ARTICLE

Potential therapeutic targets and medications for arteriovenous malformations of the central nervous system

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Abstract

Arteriovenous malformations (AVMs) of the central nervous system are high-flow arteriovenous shunts that lead to considerable risks of hemorrhagic stroke and neurological deficits in young patients. Due to the complex angioarchitecture and their close anatomical relationship with the brain and spinal cord, the management of brain and spinal AVMs is challenging. Conventional invasive treatments, including microsurgery, endovascular embolization, and stereotactic radiosurgery, are associated with considerable risks and unsatisfactory efficacy. In addition, the lack of medications for AVMs represents an unmet clinical need. In recent years, the pathogenesis of AVMs has been progressively explored. The increased understanding of the mechanisms of the formation, progression, and rupture of AVMs has opened up several potential directions for AVM pharmacotherapy. In recent years, some promising drugs targeting angiogenesis, inflammation, vessel wall integrity, and the mitogen-activated protein kinase (MAPK)-extracellular receptor kinase (ERK) signaling pathway have been tested in a series of clinical investigations. In this review, we summarize the potential mechanisms, preliminary efficacy, and side effects of the candidate medications, including bevacizumab, minocycline or doxycycline, thalidomide, and trametinib, in the treatment of brain and spinal AVMs.

Keywords: Arteriovenous malformations; Therapeutic targets; Medication; Vascular integrity; Somatic mutations

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1. Introduction

Arteriovenous malformations (AVMs) are high-flow angiopathies that are characterized by direct connections between arteries and veins[1]. Although AVM lesions have been detected in most bodily tissues, they are 20 times more likely to occur in the central nervous system (CNS), as with brain arteriovenous malformations (BAVMs) and spinal cord arteriovenous malformations (SAVMs), than in other locations[2]. Nearly 95% of cases of AVMs in the CNS are sporadic or single lesions, while the remaining cases are genetic AVM syndromes, such as hereditary hemorrhagic telangiectasia (HHT), capillary malformation-arteriovenous malformation (CM-AVM) syndrome, and neurofibromatosis[3,4].

BAVMs have an incidence of 1.3/100,000 patient-years, while the incidence of SAVMs has been reported to be 1/10th that of BAVMs[5]. These lesions are a significant cause of hemorrhagic stroke in young patients. Natural history studies have shown that the annual hemorrhagic rate of an unselected BAVM cohort is 1%–4% and approximately 5% for patients with a rupture history[6-11]. Considering that BAVMs are commonly observed in young patients aged 20 – 40 years, patients with BAVMs may harbor a significantly higher mortality risk than healthy populations[12]. Compared to BAVMs, SAVMs are characterized by a greater clinical risk[13]. They usually lead to neurological deficits, resulting from hemorrhage and venous hypertension of the spinal cord. The average age of onset of SAVM is 25 years, and its pre-treatment annual neurological deficit deterioration rate is over 30%[13]. The largest cohort study has shown that the annual hemorrhagic rate is 10% and the cumulative spinal hemorrhagic rate 4 years after the initial onset of symptoms is 32%[13].

Several interventional approaches, including microsurgery, endovascular embolization, and stereotactic radiotherapy, are used alone or in combination to obliterate AVM lesions. However, due to their complex angioarchitecture and their close anatomical relationship with brain or spinal cord tissue, the outcomes of current treatment strategies are unsatisfactory. It has been indicated that the complete obliteration rate in published BAVM cases was <50%, with a permanent treatment-related neurological deficit rate of 4 – 7%[14,15]. The invasive interventions for SAVMs are even more challenging; the permanent treatment-related clinical deterioration rate reported in literature varies between 4% and 25%[13,16,17], and <40% of patients can be cured. In addition, prospective clinical trials, such as a randomized trial of unruptured BAVMs (ARUBA), have shown that interventional therapy increases the risk of stroke or death in patients with unruptured BAVMs[6]. The lack of pharmacotherapy for AVMs represents a compelling unmet clinical need and has thus prompted a series of laboratory-based and clinical investigations in the past two decades. The aim of this review is to summarize the potential pharmacotherapy targets and their clinical outcomes in the treatment of AVMs of the central nervous system.

2. Targets and medications

2.1. Angiogenesis

Although the detailed molecular pathobiology of AVM formation and progression is unclear, the growth and enlargement of AVM lesions are inevitably associated with the proliferation of vessels[18,19]. Therefore, it has been widely recognized that angiogenesis plays a key role in AVM pathogenesis.

The process of angiogenesis is regulated by pro- and antiangiogenic factors, and an imbalance between them would drive the sprouting of endothelial cells and newly formed vasculature[20,21]. Compelling studies have indicated that vascular endothelial growth factor (VEGF) is a vital and essential pro-angiogenic molecule that stimulates and regulates angiogenesis. The previous investigations have shown that patients with BAVMs have higher plasma VEGF levels and significantly elevated expression of VEGF and VEGF receptors[22-24]. A series of animal model-based studies have confirmed that the elevation of VEGF is a prerequisite for AVM formation and the inhibition of this pathway can restrict AVM occurrence[22-26]. Therefore, the VEGF signaling pathway is expected to be a promising target for AVM pharmacotherapy.

Bevacizumab, a humanized monoclonal VEGF antibody, has been shown to effectively restrict tumor growth through its antiangiogenic effect. It was approved by the Food and Drug Administration (FDA) for the treatment of several solid cancers[27,28]. The preliminary clinical outcomes of patients with HHT who are treated with bevacizumab are seemingly promising[29,30]. Published Phase 1 and Phase 2 clinical trials of bevacizumab did not show any convincing anti-epistaxis effect in HHT patients[31,32]. In another study conducted by Olsen *et al.*, bevacizumab significantly reduced cardiac output and relieved heart failure in HHT patients with liver AVMs[33]. In addition, preclinical studies have shown that this drug could reduce the number of dysplastic vessels, as indicated by a 54% reduction in the dysplasia index in a HHT-based BAVM mouse model[34]. Pazopanib (Votrient), another VEGF inhibitor, shows a convincing anti-epistaxis effect in HHT patients[35]. Table 1 shows the different clinical studies of bevacizumab and pazopanib according to timeline.

The above favorable results have prompted the investigation of the use of bevacizumab to reduce the risk of hemorrhage in CNS AVMs. However, a recently published proof-of-concept pilot study of bevacizumab in BAVMs has shown a negative result[36]. As the first clinical investigation to evaluate bevacizumab as a treatment for sporadic BAVMs, the study recruited two patients with large (Spetzler-Martin Grade IV/V) brain AVMs who denied a history of intracranial hemorrhage (ICH). The participants received 5 mg/kg of bevacizumab every 2 weeks for 12 weeks; however, the AVM volumes observed 26 and 52 weeks after the treatment did not change compared to the baseline. Although the symptoms were stable and the patients' serum VEGF levels reduced, the results were still discouraging.

Table 1. Clinical Study of VEGF Inhibition.

Medication	Target	Publication	Phase	Dosage	Duration	Number of cases	Objective	Result	Side effect
Bevacizumab	VEGF	Simonds *et al.*, 2009[30]	-	100mg, once	Retreatment is performed when needed	19	Evaluate the effectiveness	Frequency and severity of bleeding was decreased	Not displayed
Bevacizumab	VEGF	Karnezis *et al.*, 2012[29]	-	100mg, once to 8 times,	Different for each patient	19	Evaluate treatment response	effectively for epistaxis and effective and lasted for 12 months	Not displayed
Bevacizumab	VEGF	Dupuis-Girod *et al.*, 2014[31]	1	12.5/25/50/75/100 mg, once	once	40	Investigate the tolerance, bioavailability and efficacy	This nasal spray left no detection in the serum, and shows no efficacy	No dose limiting toxicity within the dose range
Bevacizumab	VEGF	Dupuis-Girod *et al.*, 2016[32]	2/3*	25/50/75 mg	3 times, for a total duration of 4 weeks	80	Evaluate the efficacy	Epistaxis duration has no significant difference	No severe Adverse events reported
Bevacizumab	VEGF	Olsen *et al.*, 2020[33]	-	5 mg/kg of body weight	6 times, for a total duration of 3 months	2	Find out treatment result	Effective for symptoms from the liver AVMs	Vulnerable and flossy nails reported; itchy, dry skin and alopecia areata
Pazopanib	VEGF	Marie *et al.*, 2019[35]	-	50mg daily	12 weeks	7	demonstrate efficacy and safety	Decrease in epistaxis duration, > 2 gm improvement in Hgb	Bronchitis and nausea; elevated alanine aminotransferase (considered severe)

*: Termination of Phase 3 study before its launching due to previous unbeneficial results on the recommendations of the monitoring committee,
–: Phase not mentioned in the article or belonging to any of them, ALT: Alanine aminotransferase, AVM: Arteriovenous malformation, Hgb: Hemoglobin, VEGF: Vascular endothelial growth factor

Although earlier experience of bevacizumab in the treatment of encephaledema, as an adverse effect caused by radiation in patients with brain AVMs, showed nidus obliteration after the administration of the drug, the combination of Gamma Knife limited the evaluation of the efficacy of bevacizumab in terms of accuracy[37].

In addition, the anti-VEGF effect of bevacizumab is toxic to endothelial cells, which may increase the hemorrhagic risk of AVMs, as reported by Tanvetyanon *et al.*, and the side effects of the drug, such as thrombotic events, severe hypertension, and cardiac failure, are also significant[38].

2.2. Inflammation

Similar to angiogenesis, the vital role of inflammation in the progression of brain AVMs has been recognized ever since early pathological studies were performed[39]. In both human and mouse AVM samples, an upregulation in the expression of a series of inflammatory factors was observed. Furthermore, the infiltration of monocytes and granulocytes was also observed in surgical AVM samples. A recently published single-cell atlas of BAVMs has established the contribution of inflammatory processes to BAVM rupture[40].

Among various inflammatory factors, matrix metalloproteinase-9 (MMP-9) is the most concerned molecule because its activation has been shown to be associated with vascular wall instability[41,42]. The matrix metalloproteinase inhibitor doxycycline has been evaluated for abdominal aortic aneurysm treatment[43]. Since the previous studies have shown that the expression of MMP-9 is significantly upregulated in BAVM samples, theoretically, the inhibition of MMP-9 may stabilize the vasculature of AVMs and reduce the hemorrhagic risk[44].

The earliest clinical investigation of MMP-9 in AVMs was conducted by Hashimoto *et al.* in 2005[22], in which 10 patients received doxycycline (100 mg, twice a day) for 1 week before BAVM resection and four patients received placebo. Compared to placebo-treated AVMs, doxycycline-treated AVMs showed a trend of reduced MMP-9 expression[45]. In 2008, Frenzel *et al.* published the results of a pilot study of minocycline and doxycycline in the treatment

of BAVMs[46]. Twelve BAVM patients were recruited and were treated with minocycline or doxycycline (200 mg/day) for up to 2 years. The results of their follow-up suggested that patients with BAVMs are able to tolerate minocycline and doxycycline well; however, their results failed to show any trend toward potential patient benefit because one BAVM ruptured during the treatment. In another study, Burrows *et al*. used marimastat, another MMP inhibitor, for the treatment of a young girl with multiple AVMs involving the upper extremities[47]. Following the treatment with marimastat, the young girl experienced pain relief, and there was healing of bony destruction; however, newly formed AVMs were observed during the treatment. Therefore, although the rationale of anti-inflammation therapy is justified and commended, there has been no solid evidence showing that it may benefit patients with AVMs of the CNS. Table 2 shows different clinical studies of MMP inhibitors according to timeline.

2.3. Vascular integrity

The results of emerging studies have suggested that BAVM rupture is associated with a maldeveloped vascular wall structure[48-50]. Compared with healthy cerebral vasculature, the BAVMs in both human and mouse models are characterized by abnormal vessels lacking mural cells (vascular smooth muscle cells and pericytes), which are similar to engorged and expanded capillaries[49]. With insights into the physiological function of mural cells, the above pathological features of AVMs may represent impaired vascular integrity and a tendency for rupture. As expected, clinical pathological investigations have indicated that these thin-walled vessels are the source of microhemorrhage and subsequent inflammatory reaction, which has been shown to be correlated with ICH in BAVMs[51]. Therefore, the repair of vascular mural cells has been considered a potential treatment strategy for BAVMs in recent years[49,50].

This hypothesis was mainly inspired by the efficacy of thalidomide for patients with HHT, as shown in a study where the platelet-derived growth factor-B/platelet-derived growth factor receptor (PDGF-B/PDGF-R) signaling pathway was upregulated and mural cells were recruited following thalidomide intervention in mice[52]. A series of clinical investigations have confirmed that oral thalidomide could significantly protect against epistaxis and gastrointestinal hemorrhage caused by telangiectasias[53,54]. From pathological and genetic points of view, telangiectasias and AVMs are distinct lesions. However, the key pathological feature of telangiectasia is also the expansion of capillaries with pericyte coverage reduction. The previous studies have revealed that thalidomide can enhance pericyte coverage in telangiectasia by enhancing the expression of PDGF-B in endothelial cells[52]. The PDGF-B/PDGF-R signaling pathway plays a key role in recruiting mural cells. During angiogenesis, endothelial cell paracrine PDGF-B and its receptor PDGF-R are mainly expressed in the precursors of pericytes and vascular smooth muscle cells[52]. Although no compelling studies have found an impairment of the PDGFB/PDGFR signaling pathway in sporadic AVMs, the above findings still indicate that thalidomide may be a promising candidate for the pharmacological treatment of brain and spinal AVMs. To the best of our knowledge, thalidomide has not been investigated in patients with brain or spinal AVMs. A major concern about the use of thalidomide in AVM patients is its rare and frequent toxicities, such as teratogenicity and peripheral neuropathy[55]. In addition, a relapse of epistaxis

Table 2. Clinical Study of MMP Inhibitors.

Medication	Target	Publication	Phase	Dosage	Duration	Number of cases	Objective	Result	Side effect
Doxycycline	MMP-9	Hashimoto *et al*., 2005[22]	-	100 mg, twice a day	One week	14	Investigate doxycycline effect for MMP-9	decreased MMP-9 in AVM tissues	Not displayed
Minocycline Doxycycline	MMP-9	Frenzel *et al*. 2008[46]	-	100 mg, twice a day	Two years	26	Evaluate feasibility	Similar adverse event rates were recorded	Dose-limiting intolerance occurred in 31% minocycline and 23% doxycycline patients
Marimastat Doxycycline	MMP	Burrows *et al*., 2009[47]	-	30-120 mg, 100 mg, daily	Several years	1	Case report of treatment effect	Pain relieved, and recurrence of symptoms appeared	No adverse effects
Doxycycline	MMP-9	Lindeman *et al*., 2009[43]	-	50/100/300 mg, daily	2 weeks	60	Evaluate vascular inflammation level	Short and selective inhibitory effect on inflammation was recorded	Not displayed

–: Phase not mentioned in the article or belonging to any of them, AVM: Arteriovenous malformation, MMP-9: Matrix metalloproteinase-9

Table 3. Clinical Study of Thalidomide.

Medication	Target	Publication	Phase	Dosage	Duration	Number of cases	Objective	Result	Side effect
Thalidomide	PDGF-B	Lebrin *et al.*, 2010[52]	–	100 mg, once a day	One month	7	Report the drug effect	6/7 patients acquired a lower rate of epistaxis frequency	Minor side effects: mild constipation, loss of libido, drowsiness and lethargy
Thalidomide	VEGF	Ge *et al.*, 2011[54]	–	100 mg, daily	4 months	55	Investigate the long-term efficacy and safety	Thalidomide group showed effective response; VEGF were significantly reduced	No severe side effects while mild side effects were common
Thalidomide	Controversial	Invernizzi *et al.*, 2015[53]	2	50-200 mg, daily	8-12 additional weeks after response	31	Study efficacy and safety	All patients' epistaxis symptoms were decreased	non-serious effects, one patient died considering unrelated

–: Phase not mentioned in the article or belonging to any of them, PDGF-B: Platelet-derived growth factor-B, VEGF: Vascular endothelial growth factor

has been observed following a cessation of treatment with thalidomide in HTT patients[52]; therefore, the potential antihemorrhagic efficacy of thalidomide for AVMs may be nullified with the withdrawal of the drug. Table 3 shows different clinical studies of thalidomide according to timeline.

2.4. Mutation of mitogen-activated protein kinase (MAPK) pathway genes

The hereditary mechanism and the causative genes of AVM syndromes, such as HHT and CM-AVM syndrome, have been extensively investigated. However, the genetic basis of sporadic AVMs was not discovered until 2017, when Couto *et al.* performed whole-exome sequencing on 10 extracranial AVM samples and found that 7 (70%) of them harbored mutations in *mitogen-activated protein kinase kinase 1* (*MAP2K1*) in endothelial cells[56]. The results were confirmed by droplet digital polymerase chain reaction (PCR)[56]. In 2018, Nikolaev *et al.* demonstrated somatic activating *KRAS* mutations in BAVM samples and localized the mutant gene in endothelial cells[57]. Through ultra-deep next-generation sequencing of a panel of 422 common tumor genes and droplet digital PCR, our group also demonstrated a high prevalence (87.1%) of *KRAS/BRAF* somatic mutations in sporadic brain and spinal AVMs[58]. Since *Kirsten rat sarcoma viral oncogene homologue* (*KRAS*), *proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homologue B* (*BRAF*), and *MAP2K1* all belong to the MAPK signaling pathway, these studies have confirmed that there is genetic homogeneity in sporadic AVMs. Figure 1 presents the KRAS/BRAF signaling pathway and MEK1 (also known as MAP2K1) inhibitor in sporadic AVMs.

Two important animal model studies have answered the question of whether the somatic mutations in MAPK pathway genes are the cause of AVM formation or the consequence of AVM progression[59,60]. They have found that both heterozygous *KRAS* G12D mutation and the overexpression of *KRAS* G12V in cerebral endothelial cells can cause BAVM lesions in mice. More importantly, these studies have verified that the activation of ERK-MAPK signaling pathway, rather than phosphoinositide 3-kinase (PI3K)-MAPK, is the cause of AVM formation, and trametinib, an MAP2K1 inhibitor, can restrict the development and progression of AVM in animal models[60]. Therefore, a genotype-guided targeted treatment for sporadic AVMs with activating mutations of ERK-MAPK pathway genes has been developed, and trametinib has been used to treat four patients with extracranial AVMs thus far[61-64]. Although the outcomes in these studies were measured in a crude and relatively subjective manner, the results still indicated that trametinib is effective in reducing the size of and blood flow to AVM lesions. Table 4 shows different clinical studies of trametinib according to timeline.

Clinical trials of trametinib in patients with BAVMs or SAVMs have not been reported in literature. One limitation is that genotype-guided management relies on the availability of AVM samples; however, a biopsy of brain or spinal AVMs is risky and not feasible in most cases. As a countermeasure, cell-free DNA sequencing may be a promising strategy, although its sensitivity is currently not favorable. Cooke *et al.* have revealed the findings of an endovascular biopsy, using coils to collect endothelial cells of BAVMs[65]. The biopsy was inventively designed and is practical for patients with refractory BAVM lesions.

The results of recent clinical and animal model studies have suggested that trametinib may be a promising

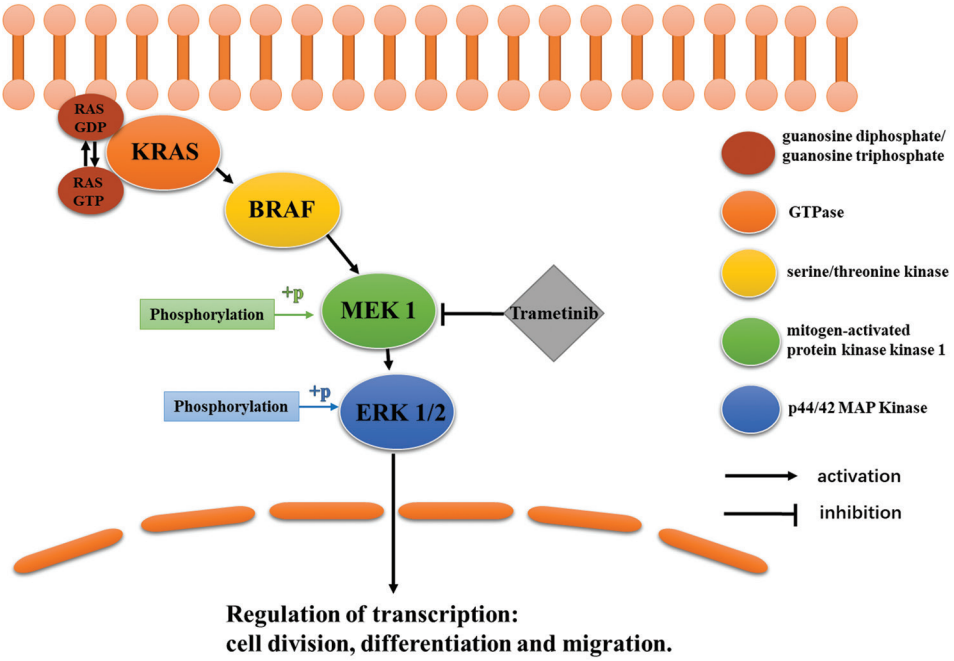


Figure 1. KRAS/BRAF signaling pathway and MEK1 inhibitor in sporadic arteriovenous malformations. Trametinib acts as a pathway inhibitor that blocks downstream responses.

Table 4. Clinical Study of Trametinib.

Medication	Target	Publication	Phase	Number of cases	Dosage	Duration	Objective	Result	Side effect
Trametinib	MEK inhibitor	Lekwuttikarn *et al.*, 2019[63]	-	1	0.5mg, daily to twice a day	6 months	Report clinical effect	Found a reduced size and lighter color	A mild acneiform eruption
Trametinib	MEK inhibitor	Edwards *et al.* 2020[61]	-	1	0.025 mg/kg, daily	6 months	Report clinical effect	75% reduction in arterial blood flow from the AVM	Not displayed
Trametinib	MEK inhibitor	Samkari *et al.*, 2021[64]	-	1	Not mentioned	6 months	Report clinical effect	No more bleeding reported, found the AVM smaller and lighter in color	Not displayed
Trametinib	MEK inhibitor	Nicholson *et al.*, 2022[62]	-	1	2.0-0.5mg, daily	Over a year, 0.5mg daily now	Report clinical effect	Shunting improved and AVM got more stable structures	Cutaneous toxicity

–: Phase not mentioned in the article or belonging to any of them, AVM: Arteriovenous malformation, MEK: Mitogen-activated protein kinase kinase

candidate for the treatment of brain and spinal AVMs[60,66]. However, in a report of a patient with Cobb syndrome, we noticed that although the extracranial AVM had shrunk after trametinib treatment, the blood flow to and lesion size of the intracranial AVM had not changed[61]. The concern about the sensitivity of brain and spinal AVMs to trametinib treatment is not groundless considering that AVMs of the CNS are quite stable compared with their extracranial counterparts[67]. In addition, considering the longer survival rate of patients with AVMs than that of patients with malignant tumors, the side effects of antineoplastic drugs should not be neglected.

3. Perspectives and conclusion

Based on the understanding of the pathogenesis and pathophysiological mechanisms of AVMs, a series of drugs targeting angiogenesis, inflammation, vascular integrity, and MAPK-ERK pathway signaling have been assumed to be effective against the disease. Unfortunately, emerging evidence has revealed that the prospects of these drugs may not be as optimistic as expected. Bevacizumab used to be the most anticipated candidate; however, there was no change observed in the structure of BAVMs in patients who received the drug. MMP-9 inhibitors were thought to

be able to stabilize the vasculature of AVMs, but it has been reported that patients under MMP inhibition treatment still carry a risk for BAVM rupture. The use of thalidomide, on the other hand, may enhance the integrity of AVM vessels and reduce the hemorrhagic risk, but its noticeable toxicities undoubtedly restrict its use. In a patient with Cobb syndrome, trametinib has shown to be effective for the treatment of extracranial AVMs but not spinal cord AVMs, indicating that AVMs of the CNS may not be sensitive to trametinib, just as the natural course of brain and spinal AVMs is more stable than that of extracranial AVMs. The safety of trametinib should also be assessed for patients without malignant diseases. Although some of the findings have not been fully translated into clinical studies, they are still of interest. The Notch signaling pathway has been the subject of a number of research, some of which have revealed that it is activated in surgical specimens and its upregulation alters the vascular structure, as shown in a mouse model of Notch-mediated AVMs[68,69]. The previous studies have also shown that members of the Notch signaling pathway and their downstream molecules are associated with angiogenesis[70,71] and that hypoxia upregulates Notch signaling, thus causing inflammation and vascular remodeling[72,73], whose subsequent reactions are related to the formation of AVM. Notch is associated with multiple pathways of conduction but its mechanism in AVM has not been fully elucidated[74,75]. The study of Notch inhibitors is challenging due to their wide range of interactions. Unfortunately, clinical studies are not yet in sight. As of now, we know that Notch inhibitor causes dose-dependent inhibition of endothelial cell migration and network formation *in vitro*[76]. A Smad family member 4 (Smad4)-inducible, endothelial cell-specific knockout (Smad4-iECKO) mouse model has established that angiopoietin-2 inhibition rescues AVM formation, which is indeed an inspiring finding[77].

We speculate that the relatively stable natural course of CNS AVMs compared to that of malignant tumors presents a challenge in the development of pharmacotherapy for AVMs. The evidence on the growth of AVMs in the brain or spinal cord is anecdotal, and the existence of arteriovenous shunts with expanded vascular caliber mainly relies on arterial blood flow rather than aberrant signaling pathways, as is the case with neoplasms. Therefore, pharmacotherapy strategies for malignant tumors may not be appropriate for AVMs. Given the applicability of the genetic-based mouse model of sporadic AVMs, we believe that further studies should be devoted to assessing the difference in expression between malformed vessels and intact vasculature. The development of specific markers may help distinguish AVM vessels from normal vessels, which may facilitate precise delivery of drugs for AVMs. In conclusion, invasive treatments will likely continue to be the sole management strategy for AVMs for a considerable amount of time, and the lack of pharmacotherapy for brain and spinal AVMs represents an unmet clinical need and, without a doubt, a significant potential for innovative research.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

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References

1. Leblanc GG, Golanov E, Awad IA, *et al.*, 2009, Biology of vascular malformations of the brain NINDS workshop collaborators. Biology of vascular malformations of the brain. *Stroke*, 40(12): 694-702.

   https://doi.org/10.1161/STROKEAHA.109.563692

2. Gomes MM, Bernatz PE, 1970, Arteriovenous fistulas: A review and ten-year experience at the Mayo clinic. *Mayo Clin Proc*, 45(2): 81-102.

3. Eerola I, Boon LM, Mulliken JB, *et al.*, 2003, Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. *Am J Hum Genet*, 73(6): 1240-1249.

   https://doi.org/10.1086/379793

4. Gallione CJ, Repetto GM, Legius E, *et al.*, 2004, A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). *Lancet*, 363(9412): 852-859.

https://doi.org/10.1016/S0140-6736(04)15732-2

5. Cogen P, Stein BM, 1983, Spinal cord arteriovenous malformations with significant intramedullary components. *J Neurosurg*, 59(3): 471-478.

https://doi.org/10.3171/jns.1983.59.3.0471

6. Mohr JP, Parides MK, Stapf C, *et al.*, 2014, Medical management with or without interventional therapy for unruptured brain arteriovenous malformations (ARUBA): A multicentre, non-blinded, randomised trial. *Lancet*, 383(9917): 614-621.

https://doi.org/10.1016/S0140-6736(13)62302-8

7. Brown RD, Wiebers DO, Forbes G, *et al.*, 1988, The natural history of unruptured intracranial arteriovenous malformations. *J Neurosurg,* 68(3): 352-357.

https://doi.org/10.3171/jns.1988.68.3.0352

8. Derdeyn CP, Zipfel GJ, Albuquerque FC, *et al.*, 2017, Management of brain arteriovenous malformations: A scientific statement for healthcare professionals from the American heart association/American stroke association. *Stroke*, 48(8): 200-224.

https://doi.org/10.1161/STR.0000000000000134

9. Al-Shahi R, 2001, A systematic review of the frequency and prognosis of arteriovenous malformations of the brain in adults. *Brain*, 124(10): 1900-1926.

https://doi.org/10.1093/brain/124.10.1900

10. Hernesniemi JA, Dashti R, Juvela S, *et al.*, 2008, Natural history of brain arteriovenous malformations: A long-term follow-up study of risk of hemorrhage in 238 patients. *Neurosurgery*, 63(5): 823-831.

https://doi.org/10.1227/01.NEU.0000330401.82582.5E

11. Kim H, Al-Shahi Salman R, McCulloch CE, *et al.*, 2014, For the MARS coinvestigators. untreated brain arteriovenous malformation: Patient-level meta-analysis of hemorrhage predictors. *Neurology*, 83(7):590-597.

https://doi.org/10.1212/WNL.0000000000000688

12. Hofmeister C, Stapf C, Hartmann A, *et al.*, 2000, Demographic, morphological, and clinical characteristics of 1289 patients with brain arteriovenous malformation. *Stroke*, 31(6): 1307-1310.

https://doi.org/10.1161/01.STR.31.6.1307

13. Yu JX, Hong T, Krings T, *et al.*, 2019, Natural history of spinal cord arteriovenous shunts: An observational study. *Brain*, 142(8): 2265-2275.

https://doi.org/10.1093/brain/awz153

14. Solomon RA, Connolly ES, 2017, Arteriovenous Malformations of the Brain. *N Engl J Med*, 376(19): 859-1866.

https://doi.org/10.1056/NEJMra1607407

15. Taylor CL, Dutton K, Rappard G, *et al.*, 2004, Complications of preoperative embolization of cerebral arteriovenous malformations. *J Neurosurg*, 100(5): 810-812.

https://doi.org/10.3171/jns.2004.100.5.0810

16. Cho WS, Kim KJ, Kwon OK, *et al.*, 2013, Clinical features and treatment outcomes of the spinal arteriovenous fistulas and malformations: Clinical article. *J Neurosurg Spine*, 19(2): 207-216.

https://doi.org/10.3171/2013.4.spine12732

17. Lee YJ, Terbrugge KG, Saliou G, *et al.*, 2014, Clinical features and outcomes of spinal cord arteriovenous malformations: Comparison between nidus and fistulous types. *Stroke*, 45(9): 2606-2612.

https://doi.org/10.1161/STROKEAHA.114.006087

18. Lawton MT, Rutledge WC, Kim H, *et al.*, 2015, Brain arteriovenous malformations. *Nat Rev Dis Primers*, 1(1): 15008.

https://doi.org/10.1038/nrdp.2015.8

19. Sturiale CL, Puca A, Sebastiani P, *et al.*, 2013, Single nucleotide polymorphisms associated with sporadic brain arteriovenous malformations: Where do we stand? *Brain*, 136(Pt 2): 665-681.

https://doi.org/10.1093/brain/aws180

20. Holash J, Maisonpierre PC, Compton D, *et al.*, 1999, Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science*, 284(5422): 1994-1998.

https://doi.org/10.1126/science.284.5422.1994

21. Jayson GC, Kerbel R, Ellis LM, *et al.*, 2016, Antiangiogenic therapy in oncology: current status and future directions. *Lancet*, 388(10043): 518-529.

https://doi.org/10.1016/S0140-6736(15)01088-0

22. Hashimoto T, Wu Y, Lawton MT, *et al.*, 2005, Coexpression of angiogenic factors in brain arteriovenous malformations. *Neurosurgery*, 56(5): 1058-1065; discussion 1058-1065.

23. Sandalcioglu IE, Wende D, Eggert A, *et al.*, 2006, Vascular endothelial growth factor plasma levels are significantly elevated in patients with cerebral arteriovenous malformations. *Cerebrovasc Dis*, 21(3): 154-158.

https://doi.org/10.1159/000090526

24. Yildirim O, Bicer A, Ozkan A, *et al.*, 2010, Expression of platelet-derived growth factor ligand and receptor in cerebral arteriovenous and cavernous malformations. *J Clin Neurosci*, 17(12): 1557-1562.

https://doi.org/10.1016/j.jocn.2010.04.028

25. Larrivée B, Prahst C, Gordon E, *et al.*, 2012, ALK1 signaling inhibits angiogenesis by cooperating with the notch pathway. *Dev Cell*, 22(3): 489-500.

https://doi.org/10.1016/j.devcel.2012.02.005

26. Del Toro R, Prahst C, Mathivet T, *et al.*, 2010, Identification

and functional analysis of endothelial tip cell-enriched genes. *Blood*, 116(19): 4025-4033.

https://doi.org/10.1182/blood-2010-02-270819

27. Wang Y, Fei D, Vanderlaan M, *et al*., 2004, Biological activity of bevacizumab, a humanized anti-VEGF antibody *in vitro*. *Angiogenesis*, 7(4): 335-345.

https://doi.org/10.1007/s10456-004-8272-2

28. Jubb AM, Harris AL, 2010, Biomarkers to predict the clinical efficacy of bevacizumab in cancer. *Lancet Oncol*, 11(12): 1172-1183.

https://doi.org/10.1016/S1470-2045(10)70232-1

29. Karnezis TT, Davidson TM, 2012, Treatment of hereditary hemorrhagic telangiectasia with submucosal and topical bevacizumab therapy. *Laryngoscope*, 122(3): 495-497.

https://doi.org/10.1002/lary.22501

30. Simonds J, Miller F, Mandel J, *et al*., 2009, The effect of bevacizumab (avastin) treatment on epistaxis in hereditary hemorrhagic telangiectasia. *Laryngoscope*, 119(5): 988-992.

https://doi.org/10.1002/lary.20159

31. Dupuis-Girod S, Ambrun A, Decullier E, *et al*., 2014, ELLIPSE study: A phase 1 study evaluating the tolerance of bevacizumab nasal spray in the treatment of epistaxis in hereditary hemorrhagic telangiectasia. *MAbs*, 6(3): 793-798.

https://doi.org/10.4161/mabs.28025

32. Dupuis-Girod S, Ambrun A, Decullier E, *et al*., 2016, Effect of bevacizumab nasal spray on epistaxis duration in hereditary hemorrhagic telangectasia: A randomized clinical trial. *JAMA*, 316(9): 934-942.

https://doi.org/10.1001/jama.2016.11387

33. Olsen LB, Kjeldsen AD, Poulsen MK, *et al*., 2020, High output cardiac failure in 3 patients with hereditary hemorrhagic telangiectasia and hepatic vascular malformations, evaluation of treatment. *Orphanet J Rare Dis*, 15(1): 334.

https://doi.org/10.1186/s13023-020-01583-6

34. Walker EJ, Su H, Shen F, *et al*., 2012, Bevacizumab attenuates VEGF-induced angiogenesis and vascular malformations in the adult mouse brain. *Stroke*, 43(7): 1925-1930.

https://doi.org/10.1161/STROKEAHA.111.647982

35. Faughnan ME, Gossage JR, Chakinala MM, *et al*., 2019, Pazopanib may reduce bleeding in hereditary hemorrhagic telangiectasia. *Angiogenesis*, 22(1): 145-155.

https://doi.org/10.1007/s10456-018-9646-1

36. Muster R, Ko N, Smith W, *et al*., 2021, Proof-of-concept single-arm trial of bevacizumab therapy for brain arteriovenous malformation. *BMJ Neurol Open*, 3(1): e000114.

https://doi.org/10.1136/bmjno-2020-000114

37. Williams BJ, Park DM, Sheehan JP, 2012, Bevacizumab used for the treatment of severe, refractory perilesional edema due to an arteriovenous malformation treated with stereotactic radiosurgery. *J Neurosurg*. 116(5): 972-977.

https://doi.org/10.3171/2012.1.JNS111627

38. Tanvetyanon T, Murtagh R, Bepler G, 2009, Rupture of a cerebral arteriovenous malformation in a patient treated with bevacizumab. *J Thorac Oncol*, 4(2):268-269.

https://doi.org/10.1097/JTO.0b013e318195a642

39. Mouchtouris N, Jabbour PM, Starke RM, *et al*., 2015, Biology of cerebral arteriovenous malformations with a focus on inflammation. *J Cereb Blood Flow Metab*, 35(2): 167-175.

https://doi.org/10.1038/jcbfm.2014.179

40. Winkler EA, Kim CN, Ross JM, *et al*., 2022, A single-cell atlas of the normal and malformed human brain vasculature. *Science*, 375(6584): eabi7377.

https://doi.org/10.1126/science.abi7377

41. Spinale FG, 2002, Matrix metalloproteinases: Regulation and dysregulation in the failing heart. *Circ Res*, 90(5): 520-530.

https://doi.org/10.1161/01.RES.0000013290.12884.A3

42. Galis ZS, Khatri JJ, 2002, Matrix metalloproteinases in vascular remodeling and atherogenesis: The good, the bad, and the ugly. *Circ Res*, 90(3):251-262.

https://doi.org/10.1161/res.90.3.251

43. Lindeman JH, Abdul-Hussien H, van Bockel JH, *et al*., 2009, Clinical trial of doxycycline for matrix metalloproteinase-9 Inhibition in patients with an abdominal aneurysm: Doxycycline selectively depletes aortic wall neutrophils and cytotoxic T cells. *Circulation*, 119(16): 2209-2216.

https://doi.org/10.1161/CIRCULATIONAHA.108.806505

44. Hashimoto T, Wen G, Lawton MT, *et al*., 2003, Abnormal expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in brain arteriovenous malformations. *Stroke*, 34(4): 925-931.

https://doi.org/10.1161/01.STR.0000061888.71524.DF

45. Hashimoto T, Matsumoto MM, Li JF, *et al*., 2005, Suppression of MMP-9 by doxycycline in brain arteriovenous malformations. *BMC Neurol*, 5(1):1.

https://doi.org/10.1186/1471-2377-5-1

46. Frenzel T, Lee CZ, Kim H, *et al*., 2008, Feasibility of minocycline and doxycycline use as potential vasculostatic therapy for brain vascular malformations: Pilot study of adverse events and tolerance. *Cerebrovasc Dis*, 25(1-2): 157-163.

https://doi.org/10.1159/000113733

47. Burrows PE, Mulliken JB, Fishman SJ, *et al*., 2009, Pharmacological treatment of a diffuse arteriovenous malformation of the upper extremity in a child. *J Craniofac Surg*, 20 Suppl 1: 597-602.

https://doi.org/10.1097/SCS.0b013e3181927f1e

48. Meng J, Okeda R, 2001, Histopathological structure of the pial arteriovenous malformation in adults: Observation by reconstruction of serial sections of four surgical specimens. *Acta Neuropathol*, 102(1):63-68.

https://doi.org/10.1007/s004010000351

49. Chen W, Guo Y, Walker EJ, *et al*., 2013, Reduced mural cell coverage and impaired vessel integrity after angiogenic stimulation in the Alk1-deficient brain. *Arterioscler Thromb Vasc Biol*, 33(2): 305-310.

https://doi.org/10.1161/ATVBAHA.112.300485

50. Baeyens N, Larrivée B, Ola R, *et al*., 2016, Defective fluid shear stress mechanotransduction mediates hereditary hemorrhagic telangiectasia. *J Cell Biol*, 214(7): 807-816.

https://doi.org/10.1083/jcb.201603106

51. Winkler EA, Birk H, Burkhardt JK, *et al*., 2018, Reductions in brain pericytes are associated with arteriovenous malformation vascular instability. *J Neurosurg*, 129(6): 1464-1474.

https://doi.org/10.3171/2017.6.JNS17860

52. Lebrin F, Srun S, Raymond K, *et al*., 2010, Thalidomide stimulates vessel maturation and reduces epistaxis in individuals with hereditary hemorrhagic telangiectasia. *Nat Med*, 16(4): 420-428.

https://doi.org/10.1038/nm.2131

53. Invernizzi R, Quaglia F, Klersy C, *et al*., 2015, Efficacy and safety of thalidomide for the treatment of severe recurrent epistaxis in hereditary haemorrhagic telangiectasia: Results of a non-randomised, single-centre, phase 2 study. *Lancet Haematol*, 2(11):e465-e473.

https://doi.org/10.1016/S2352-3026(15)00195-7

54. Ge Z, Chen H, Gao Y, *et al*., 2011, Efficacy of thalidomide for refractory gastrointestinal bleeding from vascular malformation. *Gastroenterology*, 141(5): 1629-1637.e1-4.

https://doi.org/10.1053/j.gastro.2011.07.018

55. Tseng S, Pak G, Washenik K, *et al*., 1996, Rediscovering thalidomide: A review of its mechanism of action, side effects, and potential uses. *J Am Acad Dermatol*, 35(6):969-979.

https://doi.org/10.1016/S0190-9622(96)90122-X

56. Couto JA, Huang AY, Konczyk DJ, *et al*., 2017, Somatic MAP2K1 mutations are associated with extracranial arteriovenous malformation. *Am J Hum Genet*, 100(3):546-554.

https://doi.org/10.1016/j.ajhg.2017.01.018

57. Morita H, Komuro I, 2018, Somatic activating KRAS mutations in arteriovenous malformations of the brain. *N Engl J Med*, 378(16): 1561-1562.

https://doi.org/10.1056/NEJMc1802190

58. Hong T, Yan Y, Li J, *et al*., 2019, High prevalence of KRAS/BRAF somatic mutations in brain and spinal cord arteriovenous malformations. *Brain*, 142(1): 23-34.

https://doi.org/10.1093/brain/awy307

59. Fish JE, Suarez CP, Boudreau E, *et al*., 2020, Somatic gain of kras function in the endothelium is sufficient to cause vascular malformations that require MEK but not PI3K signaling. *Circ Res*, 127(6): 727-743.

https://doi.org/10.1161/CIRCRESAHA.119.316500

60. Park ES, Kim S, Huang S, *et al*., 2021, Selective endothelial hyperactivation of oncogenic KRAS induces brain arteriovenous malformations in mice. *Ann Neurol*, 89(5): 926-941.

https://doi.org/10.1002/ana.26059

61. Edwards EA, Phelps AS, Cooke D, *et al*., 2020, Monitoring arteriovenous malformation response to genotype-targeted therapy. *Pediatrics*, 146(3):e20193206.

https://doi.org/10.1542/peds.2019-3206

62. Nicholson CL, Flanagan S, Murati M, *et al*., 2022, Successful management of an arteriovenous malformation with trametinib in a patient with capillary-malformation arteriovenous malformation syndrome and cardiac compromise. *Pediatr Dermatol*, 39(2): 316-319.

https://doi.org/10.1111/pde.14912

63. Lekwuttikarn R, Lim YH, Admani S, *et al*., 2019, Genotype-guided medical treatment of an arteriovenous malformation in a child. *JAMA Dermatol*, 155(2): 256-257.

https://doi.org/10.1001/jamadermatol.2018.4653

64. Al-Samkari H, Eng W, 2022, A precision medicine approach to hereditary hemorrhagic telangiectasia and complex vascular anomalies. *J Thromb Haemost*, 20(5): 1077-1088.

https://doi.org/10.1111/jth.15715

65. Winkler E, Wu D, Gil E, *et al*., 2022, Endoluminal biopsy for molecular profiling of human brain vascular malformations. *Neurology*, 98(16):e1637-e1647.

https://doi.org/10.1212/WNL.0000000000200109

66. Nguyen HL, Boon LM, Vikkula M, 2022, Trametinib as a promising therapeutic option in alleviating vascular defects in an endothelial KRAS-induced mouse model. *Hum Mol Genet*, ddac169.

https://doi.org/10.1093/hmg/ddac169

67. Liu AS, Mulliken JB, Zurakowski D, *et al*., 2010, Extracranial arteriovenous malformations: Natural progression and recurrence after treatment. *Plast Reconstr Surg*, 125(4): 1185-1194.

https://doi.org/10.1097/PRS.0b013e3181d18070

68. Murphy PA, Kim TN, Lu G, *et al*., 2012, Notch4 normalization reduces blood vessel size in arteriovenous malformations. *Sci Transl Med*, 4(117): 117ra8.

https://doi.org/10.1126/scitranslmed.3002670

69. ZhuGe Q, Zhong M, Zheng W, *et al*., 2009, Notch-1 signalling is activated in brain arteriovenous malformations in humans. *Brain*, 132(Pt 12): 3231-3241.

https://doi.org/10.1093/brain/awp246

70. Carmeliet P, Jain RK, 2011, Molecular mechanisms and clinical applications of angiogenesis. *Nature*, 473(7347): 298-307.

https://doi.org/10.1038/nature10144

71. Wälchli T, Wacker A, Frei K, *et al*., 2015, Wiring the vascular network with neural cues: A CNS perspective. *Neuron*, 87(2): 271-296.

https://doi.org/10.1016/j.neuron.2015.06.038

72. Gustafsson MV, Zheng X, Pereira T, *et al*, 2005, Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Dev Cell*, 9(5): 617-628.

https://doi.org/10.1016/j.devcel.2005.09.010

73. Lim CS, Kiriakidis S, Sandison A, *et al*., 2013, Hypoxia-inducible factor pathway and diseases of the vascular wall. *J Vasc Surg*, 58(1): 219-230.

https://doi.org/10.1016/j.jvs.2013.02.240

74. Benedito R, Hellström M, 2013, Notch as a hub for signaling in angiogenesis. *Exp Cell Res*, 319(9): 1281-1288.

https://doi.org/10.1016/j.yexcr.2013.01.010

75. Caliceti C, Nigro P, Rizzo P, *et al*., 2014, ROS, notch, and Wnt signaling pathways: crosstalk between three major regulators of cardiovascular biology. *Biomed Res Int*, 2014:318714.

https://doi.org/10.1155/2014/318714

76. Davis RB, Pahl K, Datto NC, *et al*., 2018, Notch signaling pathway is a potential therapeutic target for extracranial vascular malformations. *Sci Rep*, 8(1):17987.

https://doi.org/10.1038/s41598-018-36628-1

77. Crist AM, Zhou X, Garai J, *et al*., 2019, Angiopoietin-2 inhibition rescues arteriovenous malformation in a Smad4 hereditary hemorrhagic telangiectasia mouse model. *Circulation*, 139(17): 2049-2063.

https://doi.org/10.1161/CIRCULATIONAHA.118.036952

ACCSCIENCE PUBLISHING

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REVIEW ARTICLE

Brief risk rating scale: A preliminary screening and monitoring tool emphasizing individual differences for better prognosis in Alzheimer's disease

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Abstract

Over the last several decades, significant progress has been made in the diagnostic criteria of Alzheimer's disease (AD) to identify its early stages, including subjective cognitive decline and mild cognitive impairment. However, the previous research rarely took account of individual differences when evaluating AD-spectrum patients at different stages, thereby resulting in similar treatment, which was not only ineffective but also resulted in the missed window of opportunity for intervention. In this review, we propose the Brief Risk Rating Scale (BRRS), which is predominantly based on extant literature concerning AD risk factors and brain alterations, with the aim of providing a preliminary screening and monitoring tool that can facilitate the assessment of individual's risk level, the prediction and tracking of disease progression, as well as precise treatment in a timely manner. Meanwhile, due to its simplicity and ease of use, it can be widely promoted and likewise accessible to clinicians in grassroots clinics. In general, the scale comprises two parts: The original score (O) related to patients' risk factors and the variation score (V) related to brain abnormalities tested by different sequences of magnetic resonance imaging. In addition, the advantages along with its clinical application, such as introducing BRRS into cognitive training and brain stimulation, are also discussed. We conclude that BRRS positively contributes to enhancing the accuracy of clinical diagnosis and the efficiency of personalized treatment in AD-spectrum patients, with individual differences fully considered and little additional burden added. However, the weight coefficient of each item in BRRS should be thoroughly studied in future research.

Keywords: Alzheimer's disease; Guideline; Risk factor; Magnetic resonance imaging

1. Introduction

Alzheimer's disease (AD), the most prevalent cause of dementia in the elderly, is a chronic and age-related neurodegenerative disease that is characterized by progressive declines in cognitive and functional abilities. It has been proven that pathological changes and

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network damage would have had already taken place before clinical symptoms appear[1]. Therefore, there has been a growing interest in subjective cognitive decline (SCD) and mild cognitive impairment (MCI), which are both regarded as the preclinical and prodromal stages of AD[2], in recent years. Compared to MCI, in which irreversible neuronal damage has been observed[3], SCD receives more research interest for the reason that cognitive functioning is still relatively preserved through compensation or restitution of function at this stage, and patients could potentially benefit from timely interventions[4].

However, we find that individual differences, which play a crucial role in disease progression, have rarely been emphasized in the current diagnostic or therapeutic guideline. Research has suggested that not all AD-spectrum patients have structural atrophy[5] or memory impairment[6]. A variation in brain organization, the differences in regional vulnerability, and the pathologic diversity potentially result in complex AD subtypes[7]. Throughout the prolonged course of the disease, different AD subtypes may exhibit different progressing patterns that are inconsistent with the developing order observed in the guideline[5]. Besides, the previous studies have suggested that AD is heterogeneous not only in terms of its clinical manifestations at diagnosis but also with regard to the rate of disease progression[8]. Consequently, there are several limitations in the current diagnostic criteria for a comprehensive evaluation, including the etiology, pattern and speed of progression, as well as the degree of deterioration. Our investigation finds that only a number of studies took both stages and subtypes of AD patients into account when designing the group, whereas most studies considered either of them, of which the results obtained represented the mean characteristic of AD in a normal distribution. Nonetheless, it is in essence more likely to be a skewed distribution due to individual variation among patients.

In addition, despite a significant amount of time and financial expenses invested, effective methods to cure AD remain undiscovered to date; instead, they merely serve to alleviate clinical symptoms or potentially slow the disease progression to some extent[9]. Therefore, conservative medicine or therapies are predominantly used[10]. However, such standardized treatment fails to deal with the differences in clinical features among AD patients; for example, the degree of severity and urgency. Under these circumstances, patients over the mean value are at high risk of being overlooked, thus missing the optimal time window for early intervention, which not only leads to reduced diagnostic accuracy and therapeutic effectiveness but also blocks the progress in the discovery of the pathological mechanisms underlying AD progression and in seeking for curative treatment.

AD is now regarded as a continuum progressing from unnoticeable brain alterations to subsequent memory impairments that are caused by brain alterations and eventually physical disability[11]. Considering the disease as multifactorial and multidirectional (i.e., neuron, structure, and function), it has been recommended that a spectrum may be more suitable than categorical subtypes[6]. A preliminary screening and monitoring tool that can be used in coordination with the current AD guideline to assist in identifying patients who are likely to be overlooked and at a greater risk for disease progression is essential. We, therefore, propose the Brief Risk Rating Scale (BRRS), which allows the quantification of individual risk factors and existing brain alterations, so as to make full use of individual information for the sake of a better assessment of individual's risk level, the prediction and tracking of disease progression, as well as a precise treatment in a timely manner. In parallel with the continuous efforts in discovering the underlying pathological mechanisms, developing advanced diagnostic technology, and designing new pharmacological approaches, BRRS provides an economical, efficient, and accessible way to make progress in achieving the goal of preventing, stopping, and curing AD[12,13].

2. Methods

A recent systematic review and meta-analysis[14] of AD risk factors and interventions has proposed 21 evidence-based suggestions with different levels of evidence (11 with Level A and 10 with Level B) and strengths of suggestions (19 with Class I and two with Class III) on AD prevention. It is hitherto the most comprehensive and large-scale systematic review and meta-analysis, which included a total of 243 observational prospective studies and 153 randomized controlled trials, to evaluate AD risk factors and offer clinicians and stakeholders credible guidance for AD prevention. Hence, the first part of BRRS concerning individual's risk level was formulated primarily on the basis of this excellent review.

With regard to the second part, which focuses on the aberrant structural and functional brain alterations of AD-spectrum patients, PubMed and Web of Science databases were searched for related articles, published from January 2000 to December 2021. Keyword searches were conducted using the following search terms: ([SCD (Title/Abstract)] OR [subjective cognitive impairment (Title/Abstract)] OR [MCI (Title/Abstract)] OR [AD (Title/Abstract)]) AND (AD [Title/Abstract]) AND ([magnetic resonance imaging (MRI)] OR [structural

MRI] OR [functional MRI] OR [DTI]). Initially, 10,608 records were identified after deduplication according to the following inclusion criteria: (1) Studies referring to brain abnormalities at the structural and functional levels in individuals with SCD or MCI due to AD; (2) original research conducted with different MRI sequences; and (3) articles published in English with easy access to full text. After preliminary title and abstract screening and further detailed full-text assessment, 10,409 records were eliminated for reasons as follows: (1) Case reports, clinical trials, study designs, and secondary literature, such as reviews and meta-analysis; (2) research based on animal models rather than population-based data; (3) studies out of topics, that is, studies lacking neuroimaging markers or focused on other neuroimaging techniques rather than MRI (e.g., positron emission tomography, magnetoencephalography, electroencephalography, *etc.*); studies focusing on SCD or MCI caused by conditions other than AD (e.g., cerebrovascular disease, Parkinson's disease, frontotemporal dementia, Lewy body disease, epilepsy, *etc.*); studies focusing on treatment and intervention; and studies focusing on other irrelevant topics. Ultimately, a total of 199 studies were included in the study. The literature selection process is shown in detail in a flowchart (Figure 1).

3. Results

3.1. BRRS

The BRRS comprises two parts: The original score (O) and variation score (V). The sum (S) of O and V is calculated (S = O + V) to evaluate individual clinical performance or features at present and further tracing (i.e., at follow-up or after a treatment) for a better clinical diagnosis, prognosis, and intervention. If the patient's condition corresponds with the statement in the BRRS, each corresponding item is given a score of 100 points (Table 1).

The initial score (S_0) is calculated is to establish the patient's risk level before receiving any medical intervention and to identify those with high risk for deterioration from others diagnosed in the same stage. The accumulated S_n (n = number of scoring time) at follow-up enables precise tracking of disease progression. By calculating the difference between the former and later scores, for instance, S_n–S_{n-1}, the rate of deterioration is reflected, and the effectiveness of the given intervention can be evaluated. It should be noted that to obtain precise group item and total scores, non-tested items must be clearly marked in both the O and V parts.

3.2. Evaluation standards of BRRS

Part O contains items referring to the evidence-based suggestions proposed in the latest research on AD

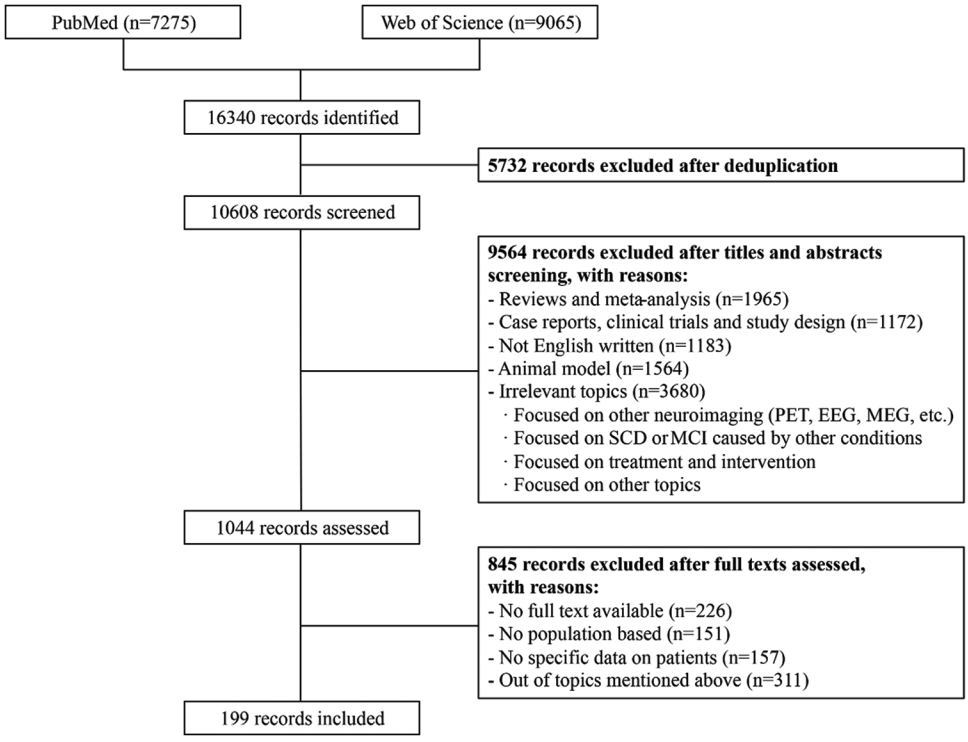


Figure 1. Flowchart of literature selection process. PET, positron emission tomography; EEG, electroencephalography; MEG, magnetoencephalography; SCD, subjective cognitive decline; MCI, mild cognitive impairment.

Table 1. Brief Risk Rating Scale.

Name: **Sex:** **Age:** **Education:** **Date:**				
Original score (O)		**Patient's score**		
		O_0	O_1	O_2
Basic information	Group score:			
	Age: >65 years (add 100 points).			
	Gender: Postmenopausal female (add 100 points).			
	Educational level: <12 years (add 100 points).			
	Pathology: (1) Detected tau protein in CSF or blood (add 100 points).			
	Pathology: (2) Detected Aβ in CSF or blood (add 100 points).			
	AD risk gene: Each gene (add 100 points).			
Lifestyle	Group score:			
	Weight: (1) Adult aged <65 years with BMI not maintained between 18.5 and 24.9 kg/m^2 (add 100 points).			
	Weight: (2) Adult aged ≥65 years with a trend of weight loss or too skinny (add 100 points).			
	History of smoking or living in a smoking environment (add 100 points).			
	Long-term history of insufficient intake of vitamin C (add 100 points).			
	Long-term history of insufficient or poor-quality sleep (add 100 points).			
	Long-term history of frailty (add 100 points).			
	Long-term history of stress (add 100 points).			
Comorbidities	Group score:			
	Diabetes history (add 100 points).			
	Head trauma history (add 100 points).			
	Cerebrovascular disease history (add 100 points).			
	Cardiovascular disease history (i.e., atrial fibrillation, hypertension, orthostatic hypotension, etc.): Each (add 100 points).			
	Depression history (add 100 points).			
	Hyperhomocysteinemia history (add 100 points).			
Variation score (V)		**Patient's score**		
		V_0	V_1	V_2
Limbic region	Group score:			
	Functional damage (add 100 points).			
	Structural damage: White matter (add 100 points).			
	Structural damage: Gray matter (add 100 points).			
	Compensation appears in function (add 100 points).			
	Compensation appears in structure (add 100 points).			
	Decompensation or the previously observed compensation disappears: Each disappearance (add 100 points).			
	WMH (add 100 points).			
Frontal region	Group score:			
	Functional damage (add 100 points).			
	Structural damage: White matter (add 100 points).			
	Structural damage: Gray matter (add 100 points).			
	Compensation appears in function (add 100 points).			
	Compensation appears in structure (add 100 points).			
	Decompensation or the previously observed compensation disappears: Each disappearance (add 100 points).			
	WMH (add 100 points).			

(*Cont'd...*)

Table 1. (*Continued*).

Name: **Sex:** **Age:** **Education:** **Date:**			
Original score (O)			**Patient's score**
			O_0 O_1 O_2
Parietal region	Group score:		
	Functional damage (add 100 points).		
	Structural damage: White matter (add 100 points).		
	Structural damage: Gray matter (add 100 points).		
	Compensation appears in function (add 100 points).		
	Compensation appears in structure (add 100 points).		
	Decompensation or the previously observed compensation disappears: Each disappearance (add 100 points).		
	WMH (add 100 points).		
Temporal region	Group score:		
	Functional damage (add 100 points).		
	Structural damage: White matter (add 100 points).		
	Structural damage: Gray matter (add 100 points).		
	Compensation appears in function (add 100 points).		
	Compensation appears in structure (add 100 points).		
	Decompensation or the previously observed compensation disappears: Each disappearance (add 100 points).		
	WMH (add 100 points).		
Occipital region	Group score:		
	Functional damage (add 100 points).		
	Structural damage: White matter (add 100 points).		
	Structural damage: Gray matter (add 100 points).		
	Compensation appears in function (add 100 points).		
	Compensation appears in structure (add 100 points).		
	Decompensation or the previously observed compensation disappears: Each disappearance (add 100 points).		
	WMH (add 100 points).		
Cerebellar region	Group score:		
	Functional damage (add 100 points).		
	Structural damage: White matter (add 100 points).		
	Structural damage: Gray matter (add 100 points).		
	Compensation appears in function (add 100 points).		
	Compensation appears in structure (add 100 points).		
	Decompensation or the previously observed compensation disappears: Each disappearance (add 100 points).		
	WMH (add 100 points).		
Total score			S_0 S_1 S_2
Note			

Aβ, beta-amyloid; BMI, body mass index; CSF, cerebrospinal fluid; WMH, white matter hyperintensity.

prevention[14,15]. It is divided into three groups: Basic information, lifestyle, and comorbidities. The basic information group consists of five items, namely, age[14], sex[16-18], education[14,18,19], AD pathological biomarkers detected in blood or cerebrospinal fluid[20-22], and AD risk genes[23-25]. The lifestyle group comprises six items that are associated with weight, diet, living environment, and sleep. The last group relates to comorbidities, such as diabetes, head trauma, cerebrovascular disease, cardiovascular disease, depression, and hyperhomocysteinemia[14]. Except for the weight item and items that have not been tested or scored points previously, there are often not too many changes in the assessment of the original score. With regard to the items that have originally scored points, despite the

improvement in the patient's condition, for example, a patient under 65 years old who scored in the weight item in S_0 but whose body mass index (BMI) has returned to normal at present, the improvement can only help to potentially slow down the rate of disease deterioration, making no difference to the reduction of the high AD risk level that has resulted from the former morbid state. Consequently, the S_n assessment of the weight items in the aforementioned case should be as follows: (1) If <65 years old but with normal BMI, no extra points will be added or deducted; (2) if over 65 years old, the weight item will be graded according to the over 65-year-old standard. As far as other items are concerned, for the same reason, once any item in O has scored points, there is no need to reappraise it at further tracing, that is, points should not be deducted, and the score should remain unchanged. Due to the minimal change in the original score regardless of whether the patient's condition becomes better or worse, it can be said to be a reliable index to assess the baseline risk level of AD patients.

Part V consists of items related to the aberrant brain alterations of AD-spectrum patients who are detected by neuroimaging techniques. As a brief scale for ease of use and promotion, we choose MRI, the most commonly used imaging modality for AD patients in current clinical practice, of difference sequences (*i.e.*, sMRI, fMRI, and diffusion tensor imaging [DTI]) for evaluation at the structural and functional levels. The evaluation standard is based on the MRI features of AD that has been comprehensively summarized from the previous studies (additional information can be found in Tables S1-S3). It has been proven that different network types and functions may exist in the same region of the brain whose alterations are not linear across the AD continuum[5,20]. Besides, the stimulation of non-invasive techniques, such as repetitive transcranial magnetic stimulation and transcranial pulse stimulation (TPS), targets the surface area of the brain and is unable to differentiate between gray and white matters or between network modules or types[13,26]. Therefore, part V focuses on six brain regions, including the limbic, frontal, parietal, temporal, and occipital regions as well as the cerebellum. Neural plasticity, which represents the adaptability and flexibility of the brain, contributes to a compensatory phenomenon against impairment in the AD spectrum, including the abnormal hyperactivation or increased connectivity in multiple brain regions[27]. This compensatory phenomenon can be viewed as a process of brain reconstruction or function remodeling to sustain optimal network functioning[28,29]; on the other hand, it can also be explained as a pathological state that may lead to further brain damage as a result of neuronal excitotoxicity[30,31]. With the progression of the disease, the compensatory ability deteriorates and eventually reaches a point of exhaustion due to extensive neurodegeneration[32]. Based on these findings, we suggest scoring each brain region by assessing the functional connectivity, structural connectivity, and compensation patterns as follows: (1) Functional damage (add 100 points); (2) structural damage of white matter (add 100 points); structural damage of gray matter (add 100 points); (3) functional compensation (add 100 points); structural compensation (add 100 points); and (4) decompensation or the disappearance of previously observed compensation (add 100 points for each disappearance). The gray and white matters are evaluated separately because there is no causal relationship in the damages between the two in AD. Meanwhile, white matter hyperintensity (WMH), which represents vascular pathology and decreasing blood flow and is considered a manifestation of cerebral small vessel disease[33,34], is associated with an increased risk of developing AD[35]. Hence, if WMH is observed, add 100 points. In every S_n evaluation, score the changes as follows: (1) Grade all changes in each brain region based on the standard and (2) no extra points added or deducted with the disappearance of WMH that was previously observed.

It is known that the development of a disease is influenced by the interactions among various factors and the modifiable network of interlocking feedback loops[12,36,37]. However, the intention of introducing the BRRS is to provide a preliminary screening and monitoring tool that can be conveniently and effectively applied in clinical settings with little additional burden added and time consumed. Therefore, we propose a method with similar items but a larger value for each item to ensure the ability to distinguish patients with a higher risk of AD progression, thus serving as a replacement for the extremely complex weighted coefficient in the interactions.

4. Discussion

4.1. Advantages and characteristic of BRRS

The BRRS may be used as a supplementary method for diagnosing AD with the following advantages: (1) Enabling objective quantification of individual differences in terms of AD risk factors and neuroimaging abnormalities; (2) enabling early identification of patients at increased risk of rapid deterioration from those diagnosed in the same stage; (3) enabling individually tailored intervention based on individual's respective scored items; (4) enabling convenient monitoring and assessment of individual's trajectories of brain changes, disease development, and therapeutic effect at follow-up; (5) reducing the time consumed and errors in calculation with the same but larger valued items, and enabling efficient judgment from

both total and individual group scores; and (6) adding insignificant burden to the diagnostic process with the ease of widespread promotion under the current AD guideline.

We recommend applying the BRRS to the AD clinical guideline in pursuit of a better understanding of AD patients. For instance, (1) if patients are in the same stage but with different S_0, more attention should be paid to those with higher scores, that is, those who are prone to deterioration; (2) if patients are in the same stage and with the same S_0, it is necessary to compare the items in each group and determine the most appropriate intervention for each patient; (3) if patients are in different stages but with the same S_0, it indicates that the patients may share similar AD risk factors and brain damage patterns despite the varying degrees of severity at the moment; the network impairment in SCD, compared with MCI and AD, is still reversible at the neuronal level; therefore, we advise implementing intensive training for SCD patients to prolong and preserve cognitive function and moderate training for MCI and AD patients in case of fluctuations in dynamic compensatory neural processes and the acceleration of the exhaustion of compensation in brain functional networks due to excessive brain activity[38]; and (4) if patients are diagnosed with different stages and S_0, both the stage and score should be considered when formulating an individually tailored intervention plan; after all, an SCD patient who scores 3000 points has a higher risk of disease progression and rapid deterioration than an AD patient who scores 100 in S_0.

4.2. Clinical application of BRRS

Although precise standards of the intensity and course of clinical treatment such as TMS, TPS, and cognitive training are still under investigation, the previous studies have proven their modulation of cortical areas or networks for compensation in the AD spectrum[39]. As a novelty, these therapeutic strategies implemented now are still in the light of the results of the previous studies and clinical experience. When designing a specialized treatment plan for individual patients with the assistance of BRRS, the total score S and the group score must not be overlooked, especially in part V. To ensure the effectiveness of treatment and prevent unnecessary intervention, the predicted improvement before every implementation must be greater than the altered S_n. With quantitative data, BRRS provides a reference for clinicians not only to set a targeted treatment protocol for patients in advance but also to evaluate the curative effect following clinical interventions. Here, we discuss the further application of BRRS in cognitive training and brain stimulation.

Cognitive training has been clinically put into practice for a period of time. It should be emphasized that cognitive differences play a key role when designing the training pattern and determining the training intensity. For example, for a patient with only memory deficits, memory training and early targeted intervention of specific brain regions are more beneficial and effective than the same standardized treatment, which can also be given to those with significant impairment in other cognitive domains. Moreover, evidence has demonstrated that trainings with higher levels of difficulty are more advantageous to patients with better performance of executive function, whereas trainings that are less challenging are more suitable for patients with poor executive performance[38]. BRRS can also help in objectively estimating patients' executive functioning.

It has been verified that high-frequency brain stimulation increases the cortical excitability of the targeted brain region, whereas low-frequency brain stimulation suppresses it[40]; additionally, there is no significant difference in the clinical effects between single stimulation and complex stimulation of multiple regions in relation to AD's known brain-affected areas[39]. Cognitive, behavioral, and functional measures can be significantly enhanced with the stimulation of the targeted brain region at a certain frequency. Consequently, we suggest formulating individual stimulation protocol based on each group score in part V of the BRRS: (1) if patients are diagnosed with only damage without any compensations in the brain region (i.e., no score in compensation items), non-excessive, effective frequency may help improve functional performance and prevent the exhaustion of compensation ability; (2) if patients have scored in compensation items (but not in decompensation), it is urgent to control the successively developing compensation with a high- or low-frequency protocol in case of exhaustion that will lead to further deterioration; (3) if patients have scored in both damage and compensation items, including structure and network (but not in decompensation), clinicians must pay close attention to the appearance of any critical patterns (e.g., brain regions continuously lose their flexibility to disease damage when modules between relevant networks gradually cluster together)[41], which indicate a high potential for decompensation; and (4) long-term intensive high-frequency stimulation has been suggested as a promising and efficient approach to rescuing the remaining well-performed function following decompensation[42,43].

4.3. Limitations of BRRS

There are still some limitations of BRRS. First, it is beyond the scope of this review to explore the weight coefficient of each item, which may to some extent affect the accuracy or credibility of BRRS. Consistent with our findings, a review has concluded that there is a preferential vulnerability of

highly selected brain regions that are primarily impaired in AD-spectrum patients, including the hippocampus, medial temporal lobe, precuneus, and temporoparietal regions[44]. Nevertheless, despite the fact that there are extensive studies on respective AD risk factors, the scarcity in comprehensive research focusing on the weight of the impact of each AD risk factor until recently makes it impractical for further discussions in this review, requiring further research to solve this in the future. In addition, although the BRRS can provide diagnostic and therapeutic guidance based on the patients' clinical performance, individual AD development rate cannot be evaluated in the early stage of application but only by means of accumulated data from the patients' total scores. Last but not least, neuropsychological testing, which we suggest to employ in conjunction with BRRS, is not included due to our consideration that it has already been widely used in clinical staging.

5. Conclusions

In this review, we propose the BRRS, a promising preliminary screening and monitoring tool based on AD risk factors and brain alteration, which can be used in parallel with the current AD guideline, with individual differences fully considered and little additional burden added. Due to its simplicity of use, the BRRS is accessible to clinicians in grassroots clinics and can be widely promoted. It has major diagnostic and therapeutic implications as it significantly contributes to realizing early and accurate identification of AD, the tracking and prognosis of AD-spectrum patients, as well as the design of individually tailored treatment in a timely manner. Furthermore, we strongly recommend that the weight coefficient of each item in BRRS be extensively studied in future research for the sake of a precise and formal application of the scale in clinical settings as soon as possible.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

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References

1. Aisen PS, Jimenez-Maggiora GA, Rafii MS, *et al*., 2022, Early-stage alzheimer disease: getting trial-ready. *Nat Rev Neurol*, 18(7): 389–399.

   https://doi.org/10.1038/s41582-022-00645-6

2. Vermunt L, Sikkes SAM, van den Hout A, *et al*., 2019, Duration of preclinical, prodromal, and dementia stages of alzheimer's disease in relation to age, sex, and APOE genotype. *Alzheimers Dement*, 15(7): 888–898.

   https://doi.org/10.1016/j.jalz.2019.04.001

3. Studart AN, Nitrini R, 2016, Subjective cognitive decline: The first clinical manifestation of alzheimer's disease? *Dement Neuropsychol*, 10(3): 170–177.

   https://doi.org/10.1590/s1980-5764-2016dn1003002

4. Rabin LA, Smart CM, Amariglio RE, 2017, Subjective cognitive decline in preclinical alzheimer's disease. *Annu Rev Clin Psychol*, 13: 369–396.

   https://doi.org/10.1146/annurev-clinpsy-032816-045136

5. Ferreira D, Pereira JB, Volpe G, *et al*., 2019, Subtypes of alzheimer's disease display distinct network abnormalities extending beyond their pattern of brain atrophy. *Front Neurol*, 10: 524.

   https://doi.org/10.3389/fneur.2019.00524

6. Lehmann M, Madison C, Ghosh PM, *et al*., 2015, Loss of functional connectivity is greater outside the default mode network in nonfamilial early-onset alzheimer's disease variants. *Neurobiol Aging*, 36(10): 2678–2686.

   https://doi.org/10.1016/j.neurobiolaging.2015.06.029

7. Vogel JW, Hansson O, 2022, Subtypes of alzheimer's disease: questions, controversy, and meaning. *Trends Neurosci*, 45(5): 342–345.

   https://doi.org/10.1016/j.tins.2022.02.001

8. Duara R, Barker W, 2022, Heterogeneity in alzheimer's disease diagnosis and progression rates: Implications for therapeutic trials. *Neurotherapeutics*, 19(1): 8–25.

   https://doi.org/10.1007/s13311-022-01185-z

9. Srivastava S, Ahmad R, Khare SK, 2021, Alzheimer's disease and its treatment by different approaches: A review. *Eur J Med Chem*, 216: 113320.

   https://doi.org/10.1016/j.ejmech.2021.113320

10. Scheltens P, Blennow K, Breteler MM, *et al*., 2016, Alzheimer's disease. *Lancet*, 388(10043): 505–517.

    https://doi.org/10.1016/s0140-6736(15)01124-1

11. 2022, 2022 alzheimer's disease facts and figures. *Alzheimers Dement*, 18(4): 700–789.

    https://doi.org/10.1002/alz.12638

12. Keine D, Walker JQ, Kennedy BK, *et al*., 2018, Development, application, and results from a precision-medicine platform that personalizes multi-modal treatment plans for mild alzheimer's disease and at-risk individuals. *Curr Aging Sci*, 11(3): 173–181.

    https://doi.org/10.2174/1874609811666181019101430

13. Sabbagh M, Sadowsky C, Tousi B, *et al*., 2020, Effects of a combined transcranial magnetic stimulation (TMS) and cognitive training intervention in patients with alzheimer's disease. *Alzheimers Dement*, 16(4): 641–650.

    https://doi.org/10.1016/j.jalz.2019.08.197

14. Yu JT, Xu W, Tan CC, *et al*., 2020, Evidence-based prevention of alzheimer's disease: Systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials. *J Neurol Neurosurg Psychiatry*, 91(11): 1201–1209.

    https://doi.org/10.1136/jnnp-2019-321913

15. Livingston G, Huntley J, Sommerlad A, *et al*., 2020, Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet*, 396(10248): 413–446.

    https://doi.org/10.1016/s0140-6736(20)30367-6

16. Malpetti M, Ballarini T, Presotto L, *et al*., 2017, Gender differences in healthy aging and alzheimer's dementia: A (18) F-FDG-PET study of brain and cognitive reserve. *Hum Brain Mapp*, 38(8): 4212–4227.

    https://doi.org/10.1002/hbm.23659

17. Rahman A, Schelbaum E, Hoffman K, *et al*., 2020, Sex-driven modifiers of alzheimer risk: A multimodality brain imaging study. *Neurology*, 95(2): e166–e178.

    https://doi.org/10.1212/wnl.0000000000009781

18. Bloomberg M, Dugravot A, Dumurgier J, *et al*., 2021, Sex differences and the role of education in cognitive ageing: Analysis of two UK-based prospective cohort studies. *Lancet Public Health*, 6(2): e106–e115.

    https://doi.org/10.1016/s2468-2667(20)30258-9

19. Perani D, Farsad M, Ballarini T, *et al*., 2017, The impact of bilingualism on brain reserve and metabolic connectivity in alzheimer's dementia. *Proc Natl Acad Sci U S A*, 114(7): 1690–1695.

    https://doi.org/10.1073/pnas.1610909114

20. Tucholka A, Grau-Rivera O, Falcon C, *et al*., 2018, Structural connectivity alterations along the alzheimer's disease continuum: Reproducibility across two independent samples and correlation with cerebrospinal fluid amyloid-β and tau. *J Alzheimers Dis*, 61(4): 1575–1587.

    https://doi.org/10.3233/jad-170553

21. Olsson B, Lautner R, Andreasson U, *et al*., 2016, CSF and blood biomarkers for the diagnosis of alzheimer's disease: A systematic review and meta-analysis. *Lancet Neurol*, 15(7): 673–684.

    https://doi.org/10.1016/s1474-4422(16)00070-3

22. Jack CR, Jr., 2022, Advances in alzheimer's disease research over the past two decades. *Lancet Neurol*, 21(10): 866–869.

    https://doi.org/10.1016/s1474-4422(22)00298-8

23. Bai F, Shi Y, Yuan Y, *et al*., 2016, Immunity factor contributes to altered brain functional networks in individuals at risk for alzheimer's disease: Neuroimaging-genetic evidence. *Brain Behav Immun*, 56: 84–95.

    https://doi.org/10.1016/j.bbi.2016.02.015

24. Hu YS, Xin J, Hu Y, *et al*., 2017, Analyzing the genes related to alzheimer's disease via a network and pathway-based approach. *Alzheimers Res Ther*, 9(1): 29.

    https://doi.org/10.1186/s13195-017-0252-z

25. Yamazaki Y, Zhao N, Caulfield TR, *et al*., 2019, Apolipoprotein E and alzheimer disease: Pathobiology and targeting strategies. *Nat Rev Neurol*, 15(9): 501–518.

    https://doi.org/10.1038/s41582-019-0228-7

26. Beisteiner R, Matt E, Fan C, *et al*., 2020, Transcranial pulse stimulation with ultrasound in alzheimer's disease-a new navigated focal brain therapy. *Adv Sci (Weinh)*, 7(3): 1902583.

    https://doi.org/10.1002/advs.201902583

27. Erk S, Spottke A, Meisen A, *et al*., 2011, Evidence of neuronal compensation during episodic memory in subjective memory impairment. *Arch Gen Psychiatry*, 68(8): 845–852.

    https://doi.org/10.1001/archgenpsychiatry.2011.80

28. Gu Y, Liu R, Qin R, *et al*., 2019, Characteristic changes in the default mode network in hypertensive patients with cognitive impairment. *Hypertens Res*, 42(4): 530–540.

    https://doi.org/10.1038/s41440-018-0176-4

29. Sheng X, Chen H, Shao P, *et al*., 2021, Brain structural network compensation is associated with cognitive impairment and alzheimer's disease pathology. *Front Neurosci*, 15: 630278.

    https://doi.org/10.3389/fnins.2021.630278

30. Hafkemeijer A, Altmann-Schneider I, Oleksik AM, *et al*.,

2013, Increased functional connectivity and brain atrophy in elderly with subjective memory complaints. *Brain Connect*, 3(4): 353–362.

https://doi.org/10.1089/brain.2013.0144

31. Mormino EC, Smiljic A, Hayenga AO, *et al*., 2011, Relationships between β-amyloid and functional connectivity in different components of the default mode network in aging. *Cereb Cortex*, 21(10): 2399–2407.

https://doi.org/10.1093/cercor/bhr025

32. Bachurin SO, Gavrilova SI, Samsonova A, *et al*., 2018, Mild cognitive impairment due to alzheimer disease: Contemporary approaches to diagnostics and pharmacological intervention. *Pharmacol Res*, 129: 216–226.

https://doi.org/10.1016/j.phrs.2017.11.021

33. Graff-Radford J, Arenaza-Urquijo EM, Knopman DS, *et al*., 2019, White matter hyperintensities: Relationship to amyloid and tau burden. *Brain*, 142(8): 2483–2491.

https://doi.org/10.1093/brain/awz162

34. Kuchtova B, Wurst Z, Mrzilkova J, *et al*., 2018, Compensatory shift of subcallosal area and paraterminal gyrus white matter parameters on DTI in patients with alzheimer disease. *Curr Alzheimer Res*, 15(6): 590–599.

https://doi.org/10.2174/1567205015666171227155510

35. Bos D, Wolters FJ, Darweesh SKL, *et al*., 2018, Cerebral small vessel disease and the risk of dementia: A systematic review and meta-analysis of population-based evidence. *Alzheimers Dement*, 14(11): 1482–1492.

https://doi.org/10.1016/j.jalz.2018.04.007

36. Del Val LP, Cantero JL, Baena D, *et al*., 2018, Damage of the temporal lobe and APOE status determine neural compensation in mild cognitive impairment. *Cortex*, 101: 136–153.

https://doi.org/10.1016/j.cortex.2018.01.018

37. Farrar DC, Mian AZ, Budson AE, *et al*., 2018, Retained executive abilities in mild cognitive impairment are associated with increased white matter network connectivity. *Eur Radiol*, 28(1): 340–347.

https://doi.org/10.1007/s00330-017-4951-4

38. Fu L, Maes JH, Kessels RP, *et al*., 2017, To boost or to CRUNCH? Effect of effortful encoding on episodic memory in older adults is dependent on executive functioning. *PLoS One*, 12(3): e0174217.

https://doi.org/10.1371/journal.pone.0174217

39. Alcalá-Lozano R, Morelos-Santana E, Cortés-Sotres JF, *et al*., 2018, Similar clinical improvement and maintenance after rTMS at 5 Hz using a simple vs. complex protocol in alzheimer's disease. *Brain Stimul*, 11(3): 625–627.

https://doi.org/10.1016/j.brs.2017.12.011

40. Sandrini M, Umiltà C, Rusconi E, 2011, The use of transcranial magnetic stimulation in cognitive neuroscience: A new synthesis of methodological issues. *Neurosci Biobehav Rev*, 35(3): 516–536.

https://doi.org/10.1016/j.neubiorev.2010.06.005

41. Sullivan MD, Anderson JAE, Turner GR, *et al*., 2019, Intrinsic neurocognitive network connectivity differences between normal aging and mild cognitive impairment are associated with cognitive status and age. *Neurobiol Aging*, 73: 219–228.

https://doi.org/10.1016/j.neurobiolaging.2018.10.001

42. Pennisi G, Ferri R, Lanza G, *et al*., 2011, Transcranial magnetic stimulation in alzheimer's disease: A neurophysiological marker of cortical hyperexcitability. *J Neural Transm (Vienna)*, 118(4): 587–598.

https://doi.org/10.1007/s00702-010-0554-9

43. Chu CS, Li CT, Brunoni AR, *et al*., 2021, Cognitive effects and acceptability of non-invasive brain stimulation on alzheimer's disease and mild cognitive impairment: A component network meta-analysis. *J Neurol Neurosurg Psychiatry*, 92(2): 195–203.

https://doi.org/10.1136/jnnp-2020-323870

44. Wang XQ, Huang WJ, Su L, *et al*., 2020, Neuroimaging advances regarding subjective cognitive decline in preclinical alzheimer's disease. *Mol Neurodegener*, 15(1): 27.

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ACCSCIENCE PUBLISHING

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REVIEW ARTICLE

Mechanism of action and therapeutic targeting of microglia in autism spectrum disorder

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Abstract

Autism spectrum disorder (ASD) is a complex mental illness with a high incidence and considerable impact. More than half of the affected individuals have self-harm behaviors, resulting in high mortality and morbidity. The impact of ASD on education and employment opportunities, the need for family care and support, as well as the burden on families and society is enormous. The underlying pathogenesis of ASD is still unclear, and effective interventions are lacking. Microglia are key immune cells in the central nervous system (CNS), and they function far beyond classical innate immunity, as they can affect normal neuronal activity by secreting cytokines and pruning synapses through phagocytosis. On the one hand, the abnormal activity of microglia may contribute to the development of ASD; on the other hand, it provides a potential target for intervention and treatment. In this review, we comprehensively analyze the mechanism of action of microglia in ASD development and summarize the current methods for targeting microglia in treating ASD.

Keywords: Autism spectrum disorder; Microglia; Neuroinflammation; Cellular immunotherapy

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1. Introduction

Autism spectrum disorder (ASD), also known as an autistic disorder, is a developmental brain disorder. The main symptoms are social handicaps, communication disorders, narrow interests, and stereotypical repetitive behaviors[1]. ASD begins before the age of three and manifests significantly thereafter. The vast majority of children require long-term rehabilitation training and special education support. One-third of individuals with ASD may remain mute all their lives, one-half have no sense of safety or need to be restrained in their actions, nearly two-thirds of children with ASD experience bullying, and approximately one-third engage in self-harm behaviors, such as head banging, arm biting, and skin scratching[2]. The average cost for individuals with ASD is approximately $60,000/year throughout their childhood[3] and increases with the onset of intellectual disability. According to the latest screening data on ASD from the Centers for Disease Control and Prevention, one in 54 children is diagnosed with ASD, with a 24% increase in prevalence from 2016 to 2018[4].

A century ago, microglia were first recognized as a distinct cell population in the central nervous system (CNS)[5] and were primarily thought to be phagocytes responsible

for removing debris during CNS development and in pathological conditions. Microglia-mediated immune inflammation was believed to affect the stability of the CNS[6]. With the advances in genetic technologies and single-cell analysis, the connection between microglia and neurons has become more apparent. Besides being central to the inherent immune response, microglia are involved in a variety of neuropsychiatric diseases; functional abnormalities of microglia have been confirmed in anxiety, depression, and other mental disorders. Therefore, the mechanism of action of microglia in mental illness is one of the trending topics in current research. The modulation of neuronal activity by microglia is evident in many brain diseases[7].

The dysfunction of microglia may be one of the underlying mechanisms in ASD[8]. Aberrant activity of microglia, abnormal levels of associated inflammatory factors, and certain cellular pathways have been observed in autistic patients and animal models. Both genetic and environmental factors that affect microglia may be involved in the etiology of ASD[9]. The deletion or amplification of certain genes may cause ASD, and changes in the peripheral environment may affect microglia, further aggravating autism-like behaviors.

In this review, we propose that microglia are related to the development and progression of ASD. Several widely accepted theories on the etiology of ASD and ASD models are discussed. We focus on the mechanistic role of microglia in ASD from the perspective of phagocytosis and synaptic pruning, which affect neuronal activity, through the release of inflammatory factors and their influence on inflammatory pathways. We also summarize several intervention approaches for ASD by modulating microglia and analyze the current challenges in treating ASD by regulating microglia in hope that this review will make modest contributions to the early diagnosis and treatment of ASD.

2. Etiology of ASD and its pathogenesis

The etiology of ASD is still unknown. Epidemiological studies, screening for many possible risk factors, could not identify a direct contributor to the development of ASD. Although the etiology of the disease is not fully understood, genetic factors, manifested as deletion or abnormal insertion of specific genes, and gene-environment interactions might lead to autistic-like behaviors. For example, genes such as *CNTNAP2*, *NLGN3*, and *SHANK3* have been shown to be associated with ASD. *Cntnap2* knockout mice, *Nlgn3* knock-in mice, and partial knockout of *Shank3* gene are the common genotypes used to construct ASD animal models. In addition, maternal immune activation (MIA) is believed to be a cause of ASD[10]. Studies have demonstrated that infection during pregnancy, especially in the first trimester, is a risk factor for the development of ASD in offspring[11]. MIA models were induced using polyriboinosinic polyribocytidylic acid (poly[I: C]), lipopolysaccharides (LPS), valproic acid treatment, simulated viral and bacterial infections, as well as other environmental factors to activate the maternal immune system[12] and test possible autistic-like behavioral manifestations in offspring (Figure 1).

Stimulation of certain physicochemical factors during pregnancy also leads to a significantly increased risk of ASD in offspring. It has been reported that exposure to valproic acid during pregnancy increases the probability of ASD in offspring by 50%[13]. Studies of the early pregnancy diets have shown that propionic acid salt might accumulate in the gastrointestinal tract of pregnant women who consume processed foods that are rich in propionate. Propionate can pass through the placental barrier, bind with G-protein receptors 41 (GPR41) on glial progenitor cells, and cause impaired neural differentiation. Furthermore, the expression of downstream phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is inhibited, and protein kinase B (PKB) pathway is activated to promote the proliferation and differentiation of glia. Subsequently, glial cells continue to produce inflammatory cytokines and release glial fibrillary acidic protein (GFAP) after maturation, thus affecting the development of the fetal nervous system.

3. Role of microglia in ASD

Microglia, the innate immune cells of the CNS, have high plasticity and can react rapidly during pathological processes. Microglia have been implicated to play a crucial role in the development of ASD associated with CNS inflammation[14]. Recent advances have shown that microglia function far beyond the classical innate immune response and are involved in various processes, such as neuronal excitation, synaptic pruning, and remodeling[15,16]. A growing number of studies have demonstrated that the biological role of microglia has a close association with ASD[17]. As early as 2005, the significant activation of microglia and astrocytes in the brains of autistic patients has been reported by Diana *et al.*[18]. Since microglia activation is the main cellular response to CNS dysfunction, it is conceivable that abnormal glial cell is inextricably linked to ASD.

3.1. Microglia mediate abnormal neuronal synapse pruning/clearing

Microglia are involved in the pruning and clearance of neuronal synapses. Rosa *et al.*[19] have reported the

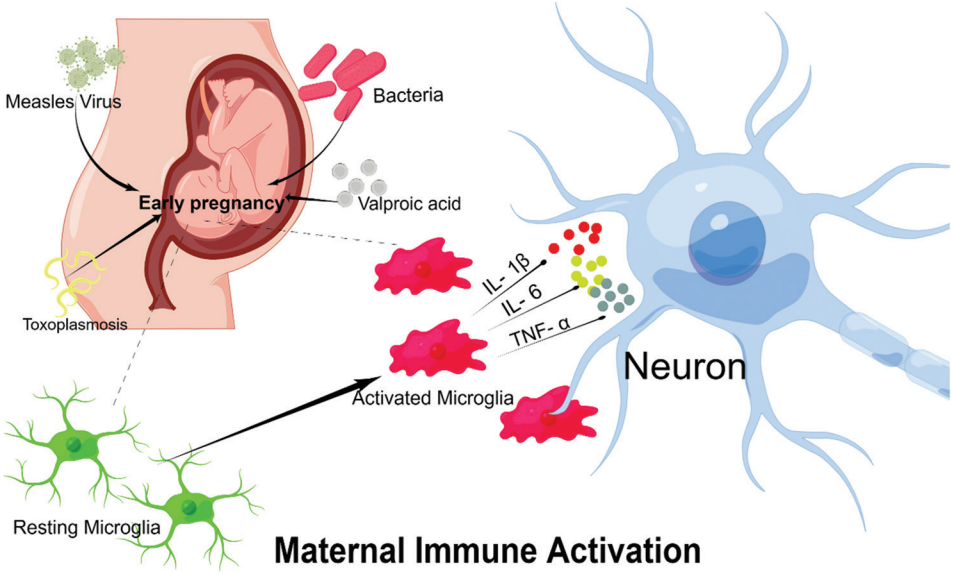


Figure 1. Maternal immune activation promotes the secretion of inflammatory cytokines through the abnormal activation of microglia. External stimulation, such as rubella virus, bacteria, and toxoplasma invasion, in the early pregnancy leads to the activation of the maternal immune system and the secretion of inflammatory cytokines through the placenta, which can activate microglia in the fetal central nervous system. Subsequently, the microglia's original branched morphology changes to amoeba-like. High levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α interact with neurons, causing aberrant neuronal functioning and ultimately resulting in autism spectrum disorder symptoms.

importance of the active engulfment of synaptic structures by microglia during neuronal development to maintain normal neuronal functions[20]. In their physiological state, microglia have a role in removing pathogens and maintaining CNS stability, directing the remodeling of brain cells after infection, removing debris in pathogenic infection, as well as restoring affected intracellular and extracellular connections. In addition, 50% of neurons undergo programmed cell death during normal brain development, and microglia dispose of these neuronal corpses through phagocytosis. However, under specific circumstances, the impairment of microglial functions, which include phagocytosis and pruning, may lead to abnormalities in neuronal function[21], thus resulting in certain autistic manifestations.

At the postsynaptic level, previous studies have identified significant neurological changes in the brain of ASD patients, such as neuronal overgrowth and microglia activation in the frontal cortex[18]. Animal experiments have revealed that autistic mice have significantly higher dendritic spine densities[22], which could be the result of pruning abnormalities and decreased microglial ability to phagocytose synapses. In addition, the impaired synaptic connections between neurons following infection, which are caused by microglia, may also contribute to ASD[23].

At the presynaptic level, several studies have shown that the balance between excitatory (E) and inhibitory (I) synapses is disrupted in ASD patients. Appropriate pruning of synapses by microglia is vital to ensure the function of excitatory and inhibitory neurons. Therefore, the aberrant pruning effect of microglia on the E/I balance may be one of the pathological reasons for ASD. It has been confirmed in animal experiments that abnormal microglial phagocytosis will break the balance of E-I synapses and eventually lead to the development of neurodevelopmental disorders and advanced brain dysfunction, such as Alzheimer's disease and ASD[24]. However, the role of E/I signaling in ASD is still controversial. Some studies have found that the function of excitatory synapses is inhibited in *Shank2*- and *Shank3*-deficient mice[25]. In contrast, other studies have shown that the synaptic E/I balance in the hippocampus inclines toward excitability[26]. Recent studies have observed the preferential elimination of excitatory synapses by microglia in pathological and physiological brains of mature rats. The impairments of microglia in pruning excitatory synapses would result in a predominance of excitatory signaling compared to inhibitory signaling in the ASD brain. The intervention of the abnormal pruning process will be a practical approach for ASD treatment in the future[16].

3.2. Microglia contribute to the progression of ASD by mediating inflammatory pathways and cytokines

Microglia are key immune cells of the CNS, and the neuroinflammation that is mediated by microglia is

centrally involved in CNS injury and disease processing. Microglia can be categorized into two phenotypes, the pro-inflammatory M1 type and the anti-inflammatory M2 type[27]. M1 microglia, which induce neurotoxicity, can secrete pro-inflammatory cytokines and chemokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. The neuroprotective M2 phenotype contributes to regulating inflammation with specific cytokines, such as IL-10 and transforming growth factor (TGF)-β, as well as neurotrophic growth factors, such as brain derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF)[28]. The balance of M1/M2 paradigm underlies different neuropathological conditions in ASD and is essential for CNS hemostasis. It is widely believed that an abnormal inflammatory response induced by M1 microglia may be an etiology of ASD. Microglia regulate neuroinflammation through specific inflammatory pathways, such as nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK), IL-1β, IL-6, IL-8, IL-17, and TNF. The dysregulation of these pathways will affect neuronal activity and result in ASD-like symptoms.

3.2.1. Inflammatory pathways

The inflammatory pathway of NF-κB that is regulated by microglia plays an important role in mediating immune response[29]. The initiation of the NF-κB signaling pathway and the transcription of cytokines involved in directing the immune response are required following an infection to clear the invading pathogenic bacteria. Therefore, the NF-κB signaling pathway is closely associated with inflammatory diseases[30] and regulates the expression of genes encoding immune response proteins[31]. Inflammatory cytokines, infection markers, or stress-activated protein kinases can induce the activation of the NF-κB pathway[32]. Within the CNS, the abnormal activation of the NF-κB signaling cascade leads to an abnormal expression of pro-inflammatory cytokines, which may disrupt normal neuronal activity[33]. A large number of literature works have reported that the activation of microglial NF-κB pathway may be involved in the regulation of ASD[34].

The MAPK signaling pathway also plays a key role in inflammation and can be activated by cytokines, stress mediators, and inflammatory markers. Microglia activation can also induce the MAPK signaling cascade, which is primarily influenced by the activation of toll-like receptors during inflammation, resulting in the phosphorylation of transcription factors[35]. Once phosphorylated, the transcription factors translocate to the nucleus and transcribe genes encoding pro-inflammatory cytokines, such as IL-6 and TNF-α, thus promoting a neuroinflammatory environment[17] and leading to an increased risk of ASD. Besides, IL-6 cytokines form a positive feedback loop by activating MAPK signaling, thus exacerbating inflammation.

3.2.2. Inflammatory cytokines

The increased release of inflammatory factors is closely associated with the development of ASD. The expression of inflammatory molecules, such as IL-1β, IL-6, IL-8, IL-17, and TNF, has been found to be elevated in ASD, and it has been suggested that the severity of the ASD phenotype might be positively correlated with the pro-inflammatory cytokine concentration[36]. Among them, pro-inflammatory cytokines IL-1β and IL-6, released by activated microglia, might have a role in neuronal damage and significantly affect ASD[37]. In addition, maternal infection or inflammatory immune activation during pregnancy may drive microglia activation[18] and promote pro-inflammatory cytokine release, which would, in turn, exacerbate the inflammatory response.

IL-1β is a key pro-inflammatory cytokine that is involved in various autoimmune inflammatory responses and cellular activities, such as cell multiplication, differentiation, and apoptosis. The synthesis and release of IL-1β are tightly regulated, and its expression is upregulated[38] in acute inflammatory, chronic inflammatory, and auto-inflammatory diseases[39]. Microglia can produce large amounts of IL-1β within the CNS[40]. Abnormal IL-1β levels may be responsible for neurological deficits of the brain in ASD. In a study of serum extracellular vesicles in children with ASD, Tsilioni *et al.*[41] found that the IL-1β levels in these children were remarkably higher than the controls following microglia activation. Another study[42] found elevated IL-1β levels in children with degenerative ASD and that IL-1β regulates the proliferation of neural precursor cells, suggesting that elevated IL-1β levels in the brain of ASD patients may alter neuronal development[43] and affect postnatal neural development[36].

IL-8 is a pro-inflammatory cytokine, secreted by various cells, including macrophages and microglia. IL-8 functions primarily as a neutrophil chemotactic factor in the blood. This pro-inflammatory cytokine that is released from microglia increases in response to pro-inflammatory stimuli. Elevated peripheral IL-8 levels have been observed in psychiatric disorders, such as major depression, bipolar affective disorder, schizophrenia, sleep disorders, ASD, anxiety disorders, and dementia[44]. In a clinical study conducted by Bryn *et al.*[45], serum IL-8 concentrations were significantly elevated in children with ASD compared to healthy controls; this finding validates the role of peripheral IL-8 associated with microglia in ASD. Another study found that ASD patients have higher IL-8 levels in the

frontal cerebral cortex[46] and that the local inflammation caused by IL-8 can affect frontal cortical processing in these patients. The frontal cortex is critical for cognitive, emotional, and social behavior[47]. Its abnormal function is closely tied to the pathological mechanisms of ASD[48] and may lead to abnormal cognitive-emotional manifestations.

IL-6 is one of the most important neuroimmune factors that are produced over a short period of time by activated microglia in response to infection and tissue injury. It promotes host defense by stimulating acute phase, hematopoietic, and immune responses[49]. IL-6 has long been shown to be associated with physiological brain development and neurological disorders, such as schizophrenia, major depression, and Alzheimer's disease[50]. Studies have shown that ASD patients have elevated IL-6 levels in their blood, which is strongly associated with neuronal cell adhesion damage, migration, and synapse formation[51]. In a study conducted by Smith *et al.*[52], ASD-like behavioral traits were found in the offspring of pregnant female rats following IL-6 injection. ASD behavior was prevented by simultaneous injection of anti-IL-6 antibodies in another experimental group, suggesting that IL-6 and abnormal microglia could influence ASD.

Using the adenoviral gene delivery approach, Wei *et al.*[53] developed a mouse model that overexpressed IL-6 centrally and confirmed that the overexpression of IL-6 is an important mediator of autistic-like behaviors. When IL-6 levels were elevated in the brains of mice, autistic-like behaviors were observed. Elevated IL-6 levels have also been reported to cause abnormal changes in the shape, length, and distribution pattern of dendritic spines, suggesting that elevated IL-6 levels may mediate ASD-like behaviors through an imbalance of neural circuits and impairment of synaptic plasticity. In the CNS, astrocytes, microglia, neurons, and endothelial cells of the cerebral microvascular system are the cellular sources of IL-6, and its levels are significantly affected by abnormal microglia activation.

3.3. Microbial-gut-brain axis and microglia

In the past few decades, the "microbial-gut-brain axis" has garnered widespread attention from researchers[54], leading to the discovery of the two-way communication between the gut and brain through pathways involving neural, endocrine, and immune systems. Intestinal microbiota is extensively involved in each pathway of the brain-gut axis, influencing neural development, cognition, and behavior by regulating the two-way communication between the gut and the CNS. Studies have shown that gut microbes play an important role in brain function[55]. Many studies have explored the effect of intestinal microbes on microglia-associated neuroinflammation and found immune abnormalities in the gastrointestinal tract of autistic patients, such as increased intestinal immunoglobulin (Ig) G, intestinal CD8 lymphocyte, and T-cell infiltration in the mucosal lamina propria. The deposition of IgG1 and IgG4 has been observed in the basement membrane of intestinal epithelium, along with an increased production of TNF-α in intestinal lymphocytes. Peripheral circulating immune cells may penetrate the blood-brain barrier (BBB) and subsequently affect neurons and glial cells, thereby perpetuating the immune response. Microglia respond to local signals within the brain and receive inputs from the periphery, including the gastrointestinal tract. There is evidence showing altered microbial community composition in nervous system diseases. Recent clinical research has revealed that gut microbes play a critical role in regulating microglial cell maturation and function[56]. However, compared with the physiological state, when mucosal rupture occurs as a result of gastrointestinal inflammation or other factors, many intestinal bacterial-related antigens, cytokines, and chemokines that damage the BBB are released continuously. The hyperactivity of the peripheral immune system promotes CNS inflammation, on which microglia are affected, thus altering the synaptic pruning of neurons and triggering ASD[57] (Figure 2).

4. Therapeutic strategies targeting microglia

In the previous section, we discuss the key roles microglia play in the neuroinflammatory process of ASD. As a dynamic cellular mediator in the CNS, microglia can disrupt the balance of the nervous system by affecting the microenvironment and deranging the expression of important neuroinflammatory molecules[27,58]. Preclinical assessment is anticipated to benefit from treatments targeting microglia dysfunction.

4.1. Microglia and inflammatory interventions

Microglia are the primary immune cells involved in neurodegenerative diseases of the CNS, such as ASD[59]. Microglia express various immune receptors that secrete numerous cytokines and chemokines. The malfunctioning of these molecules in the pathologic signaling pathways of ASD provides neuroscientists and clinicians with potential treatment targets.

4.1.1. Targeting microglia in synapse development

Insufficient pruning of weak or nonfunctional synapses by microglia can perturb neurodevelopment due to decreased levels of complement receptor 3 (CR3) or complement component 3 (C3)[60]. Microglia regulate synaptic plasticity

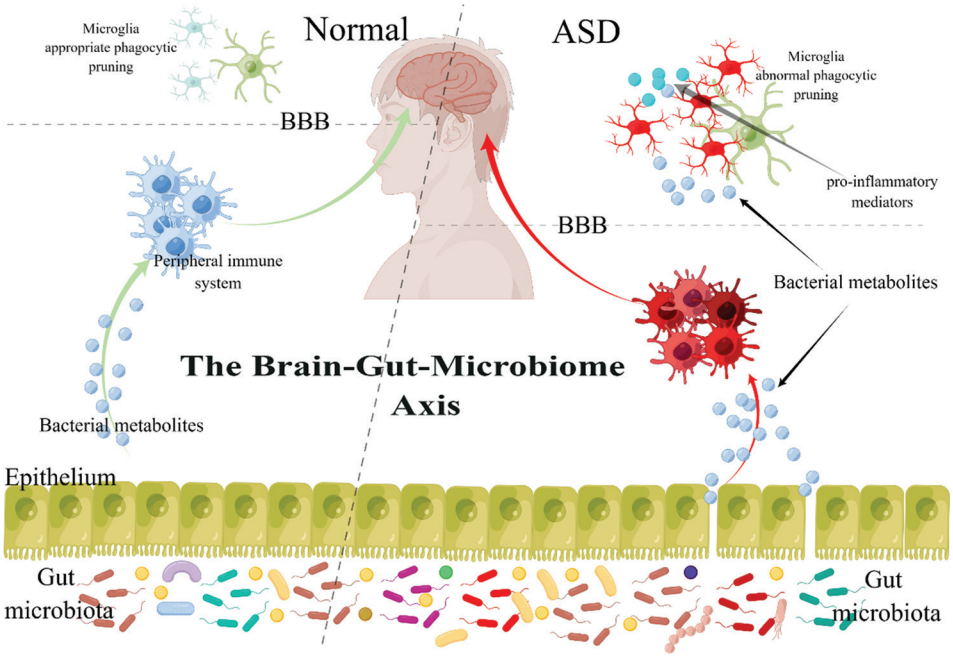


Figure 2. Involvement of microbial-gut-brain axis in autism spectrum disorder (ASD) development. The microbial-gut-brain axis plays a role in ASD development by regulating body immunity. On the left is the physiological state: the secreted metabolites from intestinal flora activate the peripheral immune system, causing the production of inflammatory factors, which reach the central nervous system through the BBB and act on microglia for normal phagocytosis and pruning. On the right is the pathological state: the integrity of intestinal epithelial cells is destroyed, and the metabolites entering the periphery are significantly increased, enhancing the peripheral immune response, and affecting the activity and physiological function of microglia.

through the secretion of reactive oxygen species (ROS) and BDNF, which engages the Trk receptors that modulate the activities of various synapses. Synaptic pruning by microglia is impaired if CX3CR1-CX3CL1 interactions are imbalanced[61]. By blocking inflammatory microglia functions, humanized antibodies or other reagents that target the aforementioned molecular markers may have beneficial effects. The inverse functions of M1/M2 microglia phenotypes provide us with a promising therapeutic idea that emphasizes on the balance of polarizations. Shifting the polarization from M1 to M2 helps in neural regeneration and regulates neuroinflammation in neurodevelopmental diseases, like ASD. The simultaneous inhibition of M1 microglia and acceleration of M2 microglia transformation might be useful for treating ASD[62]. However, due to the lack of animal experiments using M1 depressants like cyclooxygenase inhibitors, and anti-inflammatory drugs like aspirin, for treating ASD, the eligibility and availability of these drugs still require further testing. Recently, the research focus has been on microglia autophagy. It has been reported that the loss of mammalian target of rapamycin (mTOR)-dependent macro-autophagy can lead to ASD-like spine densities and a decrease in presynaptic markers, like light chain protein 3-II (LC3-II)[63]; additionally, malfunctioning autophagy can also be induced by infected microglia[64]. Hence, the potential relationship between mTOR-dependent autophagy and infected microglia may provide researchers with interesting targeting methods (Figure 3).

4.1.2. Targeting microglia in maternal immune activation

Infection during prenatal period induces pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-β, causing neuroinflammation effectors that activate microglia[65]. Coadministration of anti-IL-6 and -IL-1β antibodies has also been demonstrated in certain animal models. Suramin has shown to be effective in improving core ASD behaviors by recovering dysregulated purinergic metabolism in the poly I: C-exposed MIA model of adult ASD mice[66] (Figure 4).

4.2. Metabolic deficiency and microglia abnormalities

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are essential in maternal diet. A deficiency of n-3 PUFAs alters the offspring's microglia lipid composition and oxylipin signature, which are implicated in inflammation, resulting in microglia polarization toward a phagocytic phenotype, possibly by the overexpression of 12-hydroxyeicosatetraenoic (12-HETE)[67,68]. Phagocytic

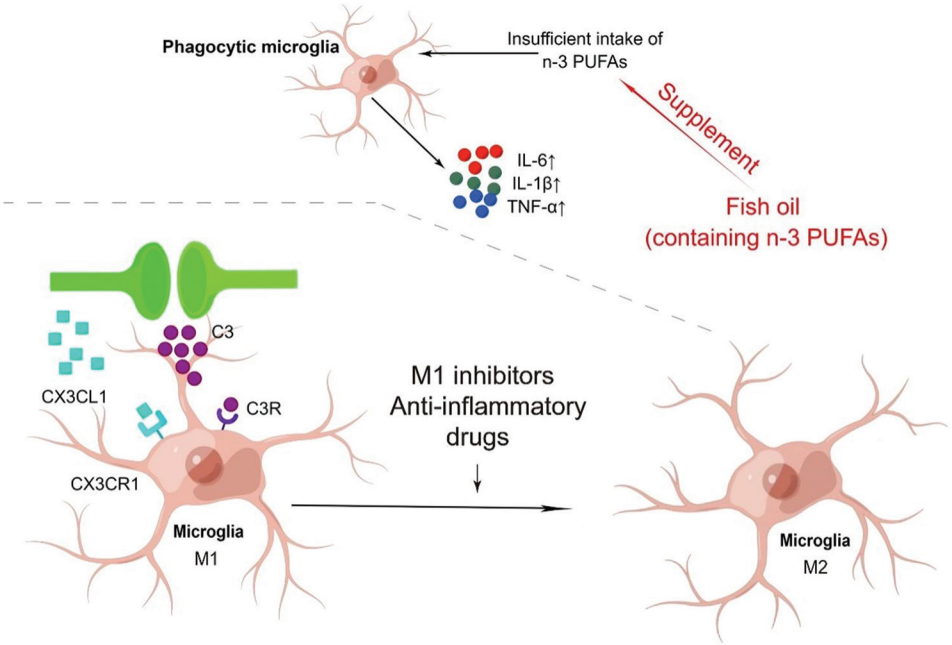


Figure 3. Treatment of autism spectrum disorder (ASD) through inflammatory and metabolic methods. Loss of omega-3 polyunsaturated fatty acids (n-3 PUFAs) leads to changes in the lipid composition and oxygen-lipid characteristics of progeny microglia, ultimately polarizing microglia toward the phagocytic phenotype and resulting in the secretion of neuroinflammatory cytokines that impair spine density and/or synaptic pruning, such as tumor necrosis factor-α, interleukin (IL)-1β, and IL-6. Supplementation of n-3 PUFAs from weaning to adulthood improves ASD-like social deficits in a mouse ASD model. Therefore, fish-oil capsules, containing n-3 fatty acids, with vitamin E supplements may be one of the key treatments for ASD patients. Shifting the polarization from M1 to M2 has potential application using different kinds of drugs.

microglia produce a cascade of neuroinflammation cytokines that impair spine refinement or synaptic pruning. Supplementing with n-3 PUFAs from weaning to adulthood has led to significant improvements in ASD-like social deficits in ASD mouse models[69]. Therefore, n-3 PUFAs supplementation through fish-oil capsules containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with vitamin E may be a pivotal treatment for ASD patients (Figure 3).

4.3. Gut-brain axis and microglia

The gut-brain axis plays an intrinsic role in brain homeostasis. Microbiome-microglia crosstalk research has generated novel therapeutic approaches for ASD patients. There have been reports of several microbiota metabolisms that impact the physiological characteristics of microglia. As bacterial metabolites derived from microbial fermentation, the three main short-chain fatty acids (SCFAs) – propionate, butyrate, and acetate – can be beneficial or detrimental for ASD treatments[63]. Using antibiotics against *Clostridia* and *Bacteroidetes*, the main producers of propionate can help prevent the increased activation of microglia. The treatment with butyrate or its byproducts generated by *Clostridium butyricum* promotes anti-inflammatory effects in microglia, contributing to the improvement of neuropsychiatric symptoms associated with ASD[70]. It has been suggested that *p*-Cresol, which is derived from *Blautia hydrogenotrophica* and *Clostridium* spp., induces microglia activation and neuroinflammation[71]. Recolonizing patients with various microbiota or feeding SCFAs can sufficiently modulate microglia activation and alleviate ASD syndrome[72].

5. Challenges

ASD is a psychosocial condition that persists throughout life. ASD patients experience social, speech, and behavioral changes that can severely disrupt their daily life and work. At present, there are many challenges in the treatment and prevention of ASD. Since the disorder cannot be cured, it becomes a lifelong problem for patients. Furthermore, the detection of ASD is not feasible at an early stage or through pregnancy screening. The diagnosis of ASD mainly relies on behavioral observations, and its symptoms cannot be detected under the age of three, thus missing the critical period of intervention.

MIA during pregnancy is one of the well-recognized mechanisms that have been successfully used to construct animal models. Altered cytokine expressions could facilitate the identification of ASD phenotypes and provide

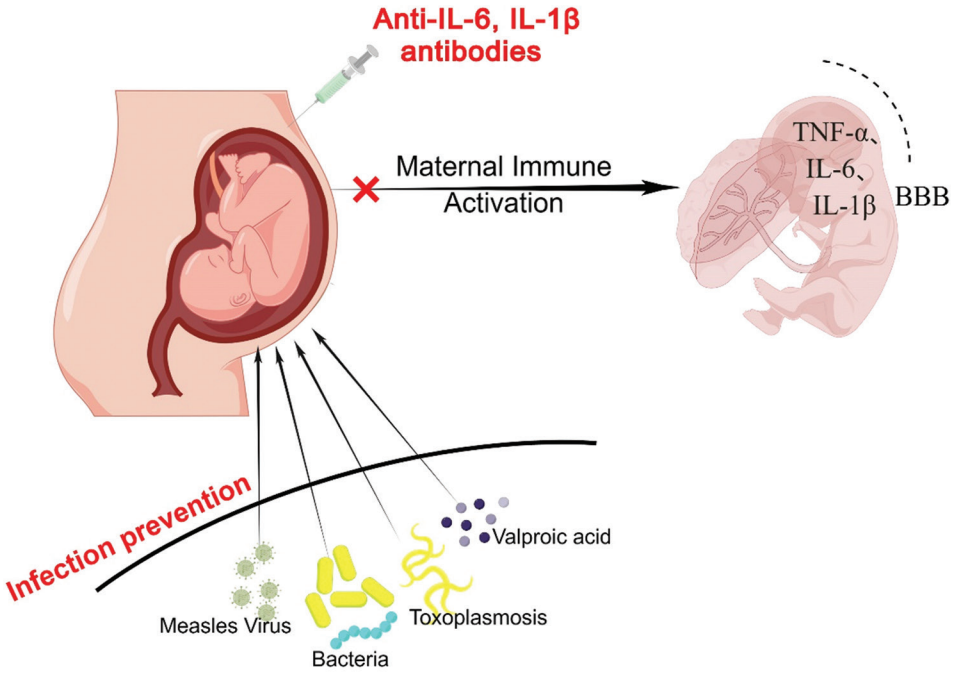


Figure 4. Targeted treatment of immune activation in pregnancy. In the early stage of pregnancy, the exposure of the mother to external stimuli, such as rubella virus, bacteria, and *Toxoplasma*, leads to the activation of microglia and the maternal immune system, as well as an increased secretion of cytokines. Treatments that are commonly used include the prevention and removal of pathogens to reduce maternal infection by stimulating the immune system and inhibiting maternal immune activation signaling; a concomitant administration of anti-interleukin (IL)-6 and -IL-1β antibodies in poly(I: C) models could take effect in animal models.

biomarkers of response to treatment[73,74]. A timely analysis of gestational samples is an excellent opportunity for pathological diagnosis and future behavioral interventions in the early ASD childhood. However, the average age of ASD children at which atypical behaviors are first noticed by the parents is 4.6 years[75]. This means that sample analysis during pregnancy may not be a suitable solution due to parental acceptance. Some animal experiments have shown promising results concerning therapeutic targeting T helper 17 (Th17) cells in preventing inflammation-induced ASD-like behaviors in offspring of susceptible mothers[76]. However, the specific inflammatory factors that cause ASD are poorly understood, and most existing immunomodulatory tools are ineffective. There are still many challenges in reversing the pathological changes in gestation to ameliorate ASD-like behaviors in offspring; however, fostering routine sample analysis and clinical trials would help address these challenges.

Microglia are innate immune cells of the CNS. An important pathogenic mechanism of ASD is the decreased ability of microglia to engulf and prune unwanted synapses, resulting in CNS inflammation. A number of studies have demonstrated that microglia release IL-1β, IL-6, IL-8, and other inflammatory factors, and these factors are significantly increased in the peripheral blood and CNS of autistic patients. Insights into possible targets associated with microglia would aid the development of potential medical therapies for various CNS disorders. A recent study identified a novel pharmacological anti-neuroinflammatory agent and GIBH-130, for Alzheimer's disease through microglia-based phenotypic screening, the method by which novel favorable targets in ASD models may be introduced[77]. Further studies are needed to confirm the specificity of inflammatory factor release and the intricate interactions between microglia and inflammation. Besides, glial cell modulation cannot be precisely controlled, and microglia modulation may have other adverse effects on the CNS, thus posing a challenge for ASD treatment.

6. Conclusion

Our work offers a summary of the main pathological mechanisms of microglia in ASD and several targeted therapeutic methods. There are limitations in the existing mechanisms, such as the ambiguous pruning preference for which type of synapses and the lack of explicit cause-effect relationship between studies, like MIA and microglia polarization. Focusing on new insights such as glial cell interactions and autophagy may bring promising results. As for effective interventions, most therapeutic methods are still

in the theoretical stage and have not been translated from ongoing animal experiments to clinical trials. The precise regulation of microglia should be the focus of future research, but elucidating the explicit circuits still remains a priority.

The emphasis of future research on ASD should include a range of relevant mechanisms: (1) the involvement of microglia in the development of ASD, and the molecular mechanisms of microglia activation, which are crucial for precise intervention; (2) the modulation of cytokines secreted by microglia, including IL-1β, IL-6, and IL-8 in both peripheral blood and CNS of ASD patients, and the inflammatory pathways they participate in, since there might be association between the role of inflammatory factors and the abnormal phagocytic pruning by microglia; (3) the characterization of microglial activity and marker molecules in the early stages of ASD, which is crucial for the early identification and intervention of ASD.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

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Availability of data

The supporting data can be obtained from the corresponding author upon reasonable request.

References

1. Maenner MJ, Shaw KA, Bakian AV, *et al*., 2021, Prevalence and characteristics of autism spectrum disorder among children Aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States 2018. *MMWR Surveill Summ*, 70(11): 1–16.

   https://doi.org/10.15585/mmwr.ss7011a1

2. Zeidan J, Fombonne E, Scorah J, *et al*., 2022, Global prevalence of autism: A systematic review update. *Autism Res*, 15(5): 778–790.

   https://doi.org/10.1002/aur.2696

3. Elsabbagh M, Divan G, Koh YJ, *et al*., 2012, Global prevalence of autism and other pervasive developmental disorders. *Autism Res*, 5(3): 160–179.

   https://doi.org/10.1002/aur.239

4. Shaw KA, Maenner MJ, Bakian AV, *et al*., 2021, Early identification of autism spectrum disorder among children Aged 4 Years - autism and developmental disabilities monitoring network, 11 sites, United States, 2018. *MMWR Surveill Summ*, 70(10): 1–14.

   https://doi.org/10.15585/mmwr.ss7010a1

5. Borst K, Dumas AA, Prinz M, 2021, Microglia: Immune and non-immune functions. *Immunity*, 54(10): 2194–2208.

   https://doi.org/10.1016/j.immuni.2021.09.014

6. Cserep C, Posfai B, Denes A, 2021, Shaping neuronal fate: Functional heterogeneity of direct microglia-neuron interactions. *Neuron*, 109(2): 222–240.

   https://doi.org/10.1016/j.neuron.2020.11.007

7. Badimon A, Strasburger HJ, Ayata P, *et al*., 2020, Negative feedback control of neuronal activity by microglia. *Nature*, 586(7829): 417–423.

   https://doi.org/10.1038/s41586-020-2777-8

8. Xu ZX, Kim GH, Tan JW, *et al*., 2020, Elevated protein synthesis in microglia causes autism-like synaptic and behavioral aberrations. *Nat Commun,* 11(1): 1797.

   https://doi.org/10.1038/s41467-020-15530-3

9. Velmeshev D, Schirmer L, Jung D, *et al*., 2019, Single-cell genomics identifies cell type-specific molecular changes in autism. *Science*, 364(6441): 685–689.

   https://doi.org/10.1126/science.aav8130

10. Elsabbagh M, 2020, Linking risk factors and outcomes in autism spectrum disorder: Is there evidence for resilience?. *BMJ*, 368: l6880.

    https://doi.org/10.1136/bmj.l6880

11. Kalish BT, Kim E, Finander B, *et al*., 2021, Maternal immune activation in mice disrupts proteostasis in the fetal brain. *Nat Neurosci*, 24(2): 204–213.

https://doi.org/10.1038/s41593-020-00762-9

12. Jensen AR, Lane AL, Werner BA, *et al*., 2022, Modern biomarkers for autism spectrum disorder: Future directions. *Mol Diagn Ther*, 26(5): 483–495.

https://doi.org/10.1007/s40291-022-00600-7

13. Seiffe A, Ramirez MF, Sempe L, *et al*., 2022, Juvenile handling rescues autism-related effects of prenatal exposure to valproic acid. *Sci Rep*, 12(1): 7174.

https://doi.org/10.1038/s41598-022-11269-7

14. De Luca SN, Soch A, Sominsky L, *et al*., 2020, Glial remodeling enhances short-term memory performance in Wistar rats. *J Neuroinflammation*, 17(1): 52.

https://doi.org/10.1186/s12974-020-1729-4

15. Barahona RA, Morabito S, Swarup V, *et al*., 2022, Cortical diurnal rhythms remain intact with microglial depletion. *Sci Rep*, 12(1): 114.

https://doi.org/10.1038/s41598-021-04079-w

16. Choudhury ME, Miyanishi K, Takeda H, *et al*., 2020, Phagocytic elimination of synapses by microglia during sleep. *Glia*, 68(1): 44–59.

https://doi.org/10.1002/glia.23698

17. Jin X, Simmons SK, Guo A, *et al*., 2020, *In vivo* perturb-seq reveals neuronal and glial abnormalities associated with autism risk genes. *Science*, 370(6520): eaaz6063.

https://doi.org/10.1126/science.aaz6063

18. Vargas DL, Nascimbene C, Krishnan C, *et al*., 2005, Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*, 57(1): 67–81.

https://doi.org/10.1002/ana.20315

19. Paolicelli RC, Bolasco G, Pagani F, *et al*., 2011, Synaptic pruning by microglia is necessary for normal brain development. *Science*, 333(6048): 1456–1458.

https://doi.org/10.1126/science.1202529

20. Donzis EJ, Tronson NC, 2014, Modulation of learning and memory by cytokines: Signaling mechanisms and long term consequences. *Neurobiol Learn Mem*, 115: 68–77.

https://doi.org/10.1016/j.nlm.2014.08.008

21. Donovan AP, Basson MA, 2017, The neuroanatomy of autism- a developmental perspective. *J Anat*, 230(1): 4–15.

https://doi.org/10.1111/joa.12542

22. Varghese M, Keshav N, Jacot-Descombes S, *et al*., 2017, Autism spectrum disorder: Neuropathology and animal models. *Acta Neuropathol*, 134(4): 537–566.

https://doi.org/10.1007/s00401-017-1736-4

23. Li Q, Zhang L, Shan H, *et al*., 2022, The immuno-behavioural covariation associated with the treatment response to bumetanide in young children with autism spectrum disorder. *Transl Psychiatry*, 12(1): 228.

https://doi.org/10.1038/s41398-022-01987-x

24. Koyama R, Ikegaya Y, 2015, Microglia in the pathogenesis of autism spectrum disorders. *Neurosci Res*, 100: 1–5.

https://doi.org/10.1016/j.neures.2015.06.005

25. Won H, Lee HR, Gee HY, *et al*., 2012, Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. *Nature*, 486(7402): 261–265.

https://doi.org/10.1038/nature11208

26. Chao HT, Chen H, Samaco RC, *et al*., 2010, Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature*, 468(7321): 263–269.

https://doi.org/10.1038/nature09582

27. Colonna M, Butovsky O, 2017, Microglia function in the central nervous system during health and neurodegeneration. *Annu Rev Immunol*, 35: 441–468.

https://doi.org/10.1146/annurev-immunol-051116-052358

28. Sanjay, Park M, Lee HJ, 2022, Roles of fatty acids in microglial polarization: Evidence from *in vitro* and *in vivo* studies on neurodegenerative diseases. *Int J Mol Sci*, 23(13): 7300.

https://doi.org/10.3390/ijms23137300

29. Gilchrist SE, Pennelli GM, Hafizi S, 2021, Gas6/TAM signalling negatively regulates inflammatory induction of GM-CSF in mouse brain microglia. *Cells*, 10(12): 3281.

https://doi.org/10.3390/cells10123281

30. Hyder U, Mccann JL, Wang J, *et al*., 2020, The ARF tumor suppressor targets PPM1G/PP2Cgamma to counteract NF-kappaB transcription tuning cell survival and the inflammatory response. *Proc Natl Acad Sci U S A*, 117(51): 32594–32605.

https://doi.org/10.1073/pnas.2004470117

31. Diamant G, Dikstein R, 2013, Transcriptional control by NF-kappaB: elongation in focus. *Biochim Biophys Acta*, 1829(9): 937–945.

https://doi.org/10.1016/j.bbagrm.2013.04.007

32. Karin M, Delhase M, 2000, The I kappa B kinase (IKK) and NF-kappa B: Key elements of proinflammatory signalling. *Semin Immunol*, 12(1): 85–98.

https://doi.org/10.1006/smim.2000.0210

33. Roberti A, Chaffey LE, Greaves DR, 2022, NF-kappaB signaling and inflammation-drug repurposing to treat inflammatory disorders? *Biology* (*Basel*), 11(3): 372.

https://doi.org/10.3390/biology11030372

34. Okun E, Griffioen KJ, Lathia JD, *et al*., 2009, Toll-like receptors in neurodegeneration. *Brain Res Rev*, 59(2): 278–292.

https://doi.org/10.1016/j.brainresrev.2008.09.001

35. Kaminska B, Gozdz A, Zawadzka M, *et al*., 2009, MAPK signal transduction underlying brain inflammation and gliosis as therapeutic target. *Anat Rec* (*Hoboken*), 292(12): 1902–1913.

https://doi.org/10.1002/ar.21047

36. Ashwood P, Krakowiak P, Hertz-Picciotto I, *et al*., 2011, Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain Behav Immun*, 25(1): 40–45.

https://doi.org/10.1016/j.bbi.2010.08.003

37. Theoharides TC, Tsilioni I, Patel AB, *et al*., 2016, Atopic diseases and inflammation of the brain in the pathogenesis of autism spectrum disorders. *Transl Psychiatry*, 6(6): e844.

https://doi.org/10.1038/tp.2016.77

38. Kaneko N, Kurata M, Yamamoto T, *et al*., 2019, The role of interleukin-1 in general pathology. *Inflamm Regen*, 39: 12.

https://doi.org/10.1186/s41232-019-0101-5

39. Li DJ, Tsai CS, Hsiao RC, *et al*., 2022, Associations between Allergic and autoimmune diseases with autism spectrum disorder and attention-deficit/hyperactivity disorder within families: A population-based Cohort study. *Int J Environ Res Public Health*, 19(8): 4503.

https://doi.org/10.3390/ijerph19084503

40. Alcala A, Osborne B, Allen B, *et al*., 2022, Toll-like receptors in the mechanism of tributyltin-induced production of pro-inflammatory cytokines, IL-1beta and IL-6. *Toxicology*, 472: 153177.

https://doi.org/10.1016/j.tox.2022.153177

41. Tsilioni I, Theoharides TC, 2018, Extracellular vesicles are increased in the serum of children with autism spectrum disorder, contain mitochondrial DNA, and stimulate human microglia to secrete IL-1beta. *J Neuroinflammation*, 15(1): 239.

https://doi.org/10.1186/s12974-018-1275-5

42. Qin Y, Cao L, Zhang J, *et al*., 2022, Whole-transcriptome analysis of serum L1CAM-captured extracellular vesicles reveals neural and glycosylation changes in autism spectrum disorder. *J Mol Neurosci*, 72(6): 1274–1292.

https://doi.org/10.1007/s12031-022-01994-z

43. Che L, Paliogiannis P, Cigliano A, *et al*., 2022, Corrigendum: Pathogenetic, prognostic, and therapeutic role of fatty acid synthase in human hepatocellular carcinoma. *Front Oncol*, 12: 874053.

https://doi.org/10.3389/fonc.2022.874053

44. Tsai SJ, 2021, Role of interleukin 8 in depression and other psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry*, 106: 110173.

https://doi.org/10.1016/j.pnpbp.2020.110173

45. Bryn V, Aass HC, Skjeldal OH, *et al*., 2017, Cytokine profile in autism spectrum disorders in children. *J Mol Neurosci*, 61(1): 1–7.

https://doi.org/10.1007/s12031-016-0847-z

46. Lin CW, Septyaningtrias DE, Chao HW, *et al*., 2022, A common epigenetic mechanism across different cellular origins underlies systemic immune dysregulation in an idiopathic autism mouse model. *Mol Psychiatry*.

https://doi.org/10.1038/s41380-022-01566-y

47. Li X, Chauhan A, Sheikh AM, *et al*., 2009, Elevated immune response in the brain of autistic patients. *J Neuroimmunol*, 207(1-2): 111–116.

https://doi.org/10.1016/j.jneuroim.2008.12.002

48. Weerasekera A, Ion-Margineanu A, Nolan G, *et al*., 2022, Subcortical brain morphometry differences between adults with autism spectrum disorder and schizophrenia. *Brain Sci*, 12(4): 439.

https://doi.org/10.3390/brainsci12040439

49. Tanaka T, Narazaki M, Kishimoto T, 2014, IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*, 6(10): a016295.

https://doi.org/10.1101/cshperspect.a016295

50. Garay PA, Mcallister AK, 2010, Novel roles for immune molecules in neural development: Implications for neurodevelopmental disorders. *Front Synaptic Neurosci*, 2: 136.

https://doi.org/10.3389/fnsyn.2010.00136

51. Guma E, Bordeleau M, Gonzalez Ibanez F, *et al*., 2022, Differential effects of early or late exposure to prenatal maternal immune activation on mouse embryonic neurodevelopment. *Proc Natl Acad Sci U S A*, 119(12): e2114545119.

https://doi.org/10.1073/pnas.2114545119

52. Smith SE, Li J, Garbett K, *et al*., 2007, Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci*, 27(40): 10695–10702.

https://doi.org/10.1523/JNEUROSCI.2178-07.2007

53. Wei H, Chadman KK, Mccloskey DP, *et al*., 2012, Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. *Biochim Biophys Acta*, 1822(6): 831–842.

https://doi.org/10.1016/j.bbadis.2012.01.011

54. Cryan JF, O'Riordan KJ, Cowan CSM, *et al*., 2019, The microbiota-gut-brain axis. *Physiol Rev*, 99(4): 1877–2013.

https://doi.org/10.1152/physrev.00018.2018

55. Sampson TR, Debelius JW, Thron T, *et al*., 2016, Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson's disease. *Cell*, 167(6): 1469–1480 e12.

https://doi.org/10.1016/j.cell.2016.11.018

56. Needham BD, Tang W, Wu WL, 2018, Searching for the gut microbial contributing factors to social behavior in rodent

models of autism spectrum disorder. *Dev Neurobiol*, 78(5): 474–499.

https://doi.org/10.1002/dneu.22581

57. Abdel-Haq R, Schlachetzki JCM, Glass CK, *et al*., 2019, Microbiome-microglia connections via the gut-brain axis. *J Exp Med*, 216(1): 41–59.

https://doi.org/10.1084/jem.20180794

58. Wright-Jin EC, Gutmann DH, 2019, Microglia as dynamic cellular mediators of brain function. *Trends Mol Med*, 25(11): 967–979.

https://doi.org/10.1016/j.molmed.2019.08.013

59. Kim JW, Hong JY, Bae SM, 2018, Microglia and autism spectrum disorder: Overview of current evidence and novel immunomodulatory treatment options. *Clin Psychopharmacol Neurosci*, 16(3): 246–252.

https://doi.org/10.9758/cpn.2018.16.3.246

60. Yuan T, Orock A, Greenwood-Vanmeerveld B, 2022, An enriched environment reduces chronic stress-induced visceral pain through modulating microglial activity in the central nucleus of the amygdala. *Am J Physiol Gastrointest Liver Physiol*, 322(2): G223–G233.

https://doi.org/10.1152/ajpgi.00307.2021

61. Subbarayan MS, Joly-Amado A, Bickford PC, *et al*., 2022, CX3CL1/CX3CR1 signaling targets for the treatment of neurodegenerative diseases. *Pharmacol Ther*, 231: 107989.

https://doi.org/10.1016/j.pharmthera.2021.107989

62. Yang Z, Liu B, Yang LE, *et al*., 2019, Platycodigenin as potential drug candidate for Alzheimer's disease via modulating microglial polarization and neurite regeneration. *Molecules*, 24(18): 3207.

https://doi.org/10.3390/molecules24183207

63. Davoli-Ferreira M, Thomson CA, Mccoy KD, 2021, Microbiota and microglia interactions in ASD. *Front Immunol*, 12: 676255.

https://doi.org/10.3389/fimmu.2021.676255

64. Alirezaei M, Kiosses WB, Flynn CT, *et al*., 2008, Disruption of neuronal autophagy by infected microglia results in neurodegeneration. *PLoS One*, 3(8): e2906.

https://doi.org/10.1371/journal.pone.0002906

65. Estes ML, Mcallister AK, 2016, Maternal immune activation: Implications for neuropsychiatric disorders. *Science*, 353(6301): 772–777.

https://doi.org/10.1126/science.aag3194

66. Naviaux JC, Schuchbauer MA, Li K, *et al*., 2014, Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy. *Transl Psychiatry*, 4: e400.

https://doi.org/10.1038/tp.2014.33

67. Martinat M, Rossitto M, Di Miceli M, *et al*., 2021, Perinatal dietary polyunsaturated fatty acids in brain development, role in neurodevelopmental disorders. *Nutrients*, 13(4): 1185.

https://doi.org/10.3390/nu13041185

68. Rey C, Nadjar A, Joffre F, *et al*., 2018, Maternal n-3 polyunsaturated fatty acid dietary supply modulates microglia lipid content in the offspring. *Prostaglandins Leukot Essent Fatty Acids*, 133: 1–7.

https://doi.org/10.1016/j.plefa.2018.04.003

69. Van Elst K, Brouwers JF, Merkens JE, *et al*., 2019, Chronic dietary changes in n-6/n-3 polyunsaturated fatty acid ratios cause developmental delay and reduce social interest in mice. *Eur Neuropsychopharmacol*, 29(1): 16–31.

https://doi.org/10.1016/j.euroneuro.2018.11.1106

70. Wenzel TJ, Gates EJ, Ranger AL, *et al*., 2020, Short-chain fatty acids (SCFAs) alone or in combination regulate select immune functions of microglia-like cells. *Mol Cell Neurosci*, 105: 103493.

https://doi.org/10.1016/j.mcn.2020.103493

71. Gabriele S, Sacco R, Altieri L, *et al*., 2016, Slow intestinal transit contributes to elevate urinary p-cresol level in Italian autistic children. *Autism Res*, 9(7): 752–759.

https://doi.org/10.1002/aur.1571

72. Vuong HE, Hsiao EY, 2017, Emerging roles for the Gut microbiome in autism spectrum disorder. *Biol Psychiatry*, 81(5): 411–423.

https://doi.org/10.1016/j.biopsych.2016.08.024

73. Comi AM, Zimmerman AW, Frye VH, *et al*., 1999, Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. *Child Neurol*, 14(6): 388–394.

https://doi.org/10.1177/088307389901400608

74. Jones KL, Croen LA, Yoshida CK, *et al*., 2017, Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. *Mol Psychiatry*, 22(2): 273–279.

https://doi.org/10.1038/mp.2016.77

75. Crane L, Chester JW, Goddard L, *et al*., 2016, Experiences of autism diagnosis: A survey of over 1000 parents in the United Kingdom. *Autism*, 20(2): 153–162.

https://doi.org/10.1177/1362361315573636

76. Choi GB, Yim YS, Wong H, *et al*., 2016, The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science*, 351(6276): 933–939.

https://doi.org/10.1126/science.aad0314

77. Zhou W, Zhong G, Fu S, *et al*., 2016, Microglia-based phenotypic screening identifies a novel inhibitor of neuroinflammation effective in Alzheimer's disease models. *ACS Chem Neurosci*, 7(11): 1499–1507.

https://doi.org/10.1021/acschemneuro.6b00125

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CASE REPORT

Vertigo or dizziness as the first presentation of epilepsy: A case report

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Abstract

Vertigo or dizziness is a common neurological symptom, but it is rarely regarded as the first presenting symptom of epilepsy. Patients who present with vertigo as the first symptom of epilepsy may be misdiagnosed or missed diagnose. We report a case presenting with vertigo or dizziness as the first manifestation of epilepsy and review relevant literature. A 71-year-old female patient sought medical attention in view of paroxysmal dizziness for a year that progressively worsened over the course of 1 week. Initially, her dizziness occurred 2 or 3 times a month, lasting for less than a minute each time. However, her attacks became more frequent and severe over the course of 1 week. Twenty-four-hour electroencephalogram (EEG) monitoring showed spike-and-slow-wave complex discharges at both sphenoidal electrodes, with the left being more prominent. The patient was treated with oral valproic acid. After a 9-month follow-up, the frequency of dizziness was markedly reduced. Although the pathogenesis of epileptic vertigo remains ambiguous, the current literature indicates that vestibular cortical neurons may be closely associated with periodic abnormal discharge of the brain lobe. Based on comprehensive medical history and EEG results, we should exercise caution when diagnosing this kind of epilepsy.

Keywords: Epilepsy; Vertigo; Dizziness; Electroencephalogram

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1. Background

Vertigo or dizziness is a common neurological symptom that is related to peripheral and/or central nervous system disorders. The vestibular system extends from the peripheral vestibular part of the inner ear to the brainstem and cerebral cortex. Any lesion throughout the entire tract could lead to dizziness or vertigo. A growing number of literature has suggested that vertigo or dizziness is deemed as one of the clinical manifestations of epileptic seizures[1]. Especially the focal and intermittent epileptic discharges, which are accompanied by episodic vestibular symptoms[2], have been referred to as vertiginous seizures, epileptic vertigo or dizziness (EVD), vestibular epilepsy or seizures, and so on[3-6]. Herein, we report a case presenting with vertigo or dizziness as the first manifestation of epilepsy and review relevant literature.

2. Case presentation

A 71-year-old female patient presented to our hospital in August 2016 in view of paroxysmal dizziness for a year that progressively worsened over a week, accompanied

by heavy-headedness, floating sensation, and instability when walking. Originally, her dizziness would occur 2 or 3 times a month, with each episode lasting for less than a minute. However, over a course of 1 week, she experienced more frequent (≥5 times a day) and severe episodes of vertigo. Her symptoms were unpredictable and unrelated to position change. She did not experience any intense spinning sensation during the onset of vertigo, nor was the dizziness accompanied by hallucination, unconsciousness, headache, orbital discomfort, sweating, nausea, vomiting, abdominal pain, or diarrhea. She had no flustered feelings, chest tightness, palpitations, pruritus over her abdomen, or abdominal discomfort, including "ascending epigastric sensation." There were no concurrent neurological, ophthalmic, or auditory disorders. She did not experience agoraphobia, panic attacks, or any recent stressful situations. She had a previous history of lumbar disk herniation more than 4 years ago, and she denied history of hypertension, coronary heart disease, and diabetes. She had no history of motion sickness, migraine, trauma, surgery, or diseases that resulted in epileptic seizures. Personal history and family history were insignificant. Physical examination showed that her general condition was good. Cardiovascular, respiratory, abdominal, and nervous system examinations were normal. No abnormalities observed in vestibular and cerebellar function examinations. Her temperature and hearing examination were normal. The laboratory investigation results were as follows: C-reactive protein (CRP), liver function, kidney function, blood lipid, blood glucose, electrolytes, homocysteine, thyroid function, erythrocyte sedimentation rate (ESR), coagulation function, protein S, protein C, antinuclear antibodies (ANAs), antiphospholipid antibodies, and antiganglioside antibodies were all within the normal range. Serologic tests for human immunodeficiency virus (HIV), hepatitis B/C, and cytomegalovirus were all negative. There were no obvious abnormalities in chest radiograph, echocardiogram, abdominal ultrasound, and electrocardiogram examinations. Brain magnetic resonance imaging (MRI) showed old lacunar cerebral infarction lesions. Evaluation of intracranial and extracranial large vessels such as carotid ultrasound, computerized tomography angiography (CTA), and magnetic resonance angiography (MRA) did not show obvious stenosis nor atherosclerotic plaque. Hyperventilation did not trigger the onset of the dizziness, and no spontaneous nystagmus was observed during the episode. On otolaryngology examination, her ear canals and eardrums were normal. In addition, tests for benign paroxysmal positional vertigo (BPPV) such as bilateral Dix-Hallpike maneuver, supine roll test, Unterberger test, bilateral Dix-Hallpike maneuver, supine roll test, Unterberger test, fistula test, video head impulse test, head thrust test, and caloric reflex test were all negative. Twenty-four-hour electroencephalogram (EEG) monitoring showed spike and slow wave complex discharges occurring at both sphenoidal electrodes, with the left being more prominent (Figure 1). Oral sodium valproate (500 mg/day) was administered and the dosage was increased to 1000 mg/day after a week. A 9-month follow-up showed that the patient had no further episodes of paroxysmal dizziness.

3. Discussion

Epileptic vertigo is a rare form of epilepsy. The incidence of epilepsy presenting with vertigo or dizziness is only about 8.5%. Especially, the sole symptom of vertigo or dizziness, which is rarely reported in the literature, only occurs in approximately 0.8% of all epilepsy[3]. Its incidence is higher in children and adolescents compared with adults, especially in female[7]. Due to the differences in race, sample size, etiology, and evaluation criteria between different studies[3], a complete and consistent criterion for epileptic vertigo has yet to be established.

Although the pathogenesis of epileptic vertigo remains elusive, the current literature has indicated that vestibular cortical neurons are associated with periodic abnormal discharge of the brain lobe[8,9]. Vestibular symptoms may also occur as a result of the permanent damage of the vestibular cortical area due to chronic epilepsy[10]. The hallmarks of epilepsy, including paroxysmal, transient, stereotypical, and repetitive, are also present in epileptic vertigo. Its main clinical manifestations are recurrent attacks of dizziness and vertigo, accompanied by the rotation of the visual scene, with or without nausea, vomiting, pallor, sweating, palpitation, headache, and so on. These symptoms are unpredictable and are not associated with positional variation. With each episode, there is no loss of consciousness. Some patients may present with peripheral illusion sensation, such as rotating, drifting, tilting, swaying, heavy headedness, and unsteadiness while walking.

Each attack varies from several seconds to minutes without an exact time. However, studies have reported that the duration of almost all temporal lobe-associated vertigos usually lasts less than a minute, whereas that of non-temporal lobe-associated vertigo lasts longer[3]. This may indicate that the temporal lobe is associated with transient epileptic vertigo. Hence, missed diagnosis or misdiagnosis with transient ischemic attacks (TIA), vestibular migraine, Meniere's disease, BPPV, vestibular paroxysmia, hypoglycemia, arrhythmia, panic attacks, neurosis, somatization, or psychogenic dizziness may likely occur in such patients. In EEG, abnormalities are mainly observed in the frontal, temporal, and top area of

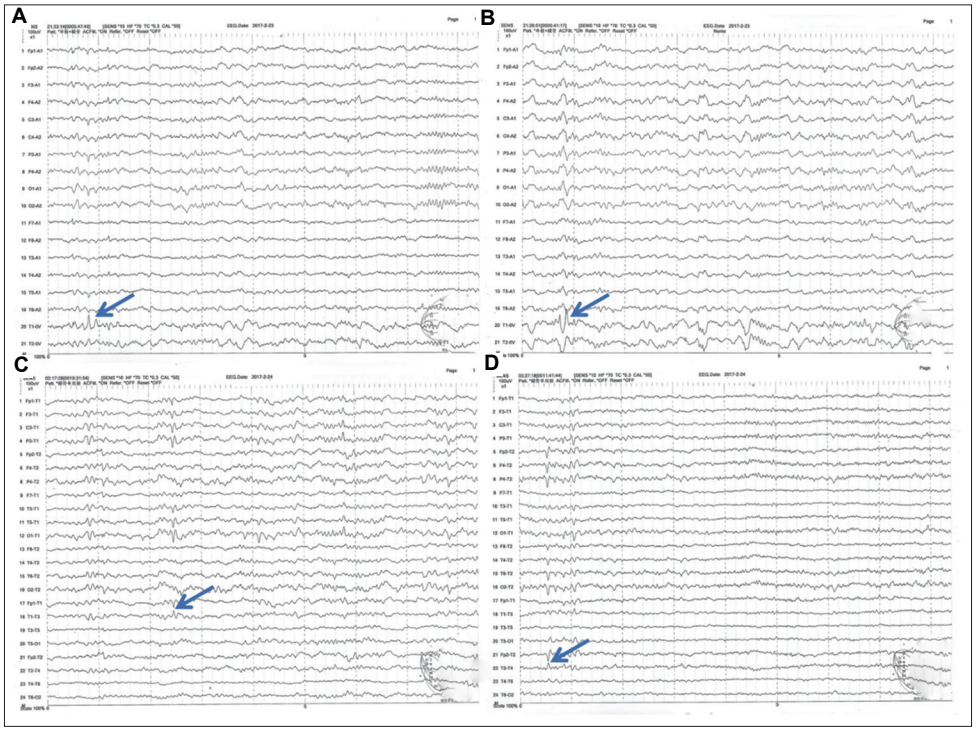


Figure 1. Electroencephalogram findings. **(A–B)** EEG shows paroxysmal spike and slow wave complex discharges in both temporal regions, with the left being more prominent. **(C)** EEG shows phase reversal in the T1 lead, located at the left temporal region. **(D)** EEG shows phase reversal in the T2 lead, located at the right temporal region. These epileptic waves are marked by the blue arrows.

the brain. These areas, especially the temporal lobe, release paroxysmal spikes or sharp waves, spike and slow wave complexes, or high amplitude slow waves[1,11].

The diagnosis of epileptic vertigo still remains a clinical problem. The incomplete medical history and the absence of ancillary examinations increase the rate of missed diagnosis and misdiagnosis of this disease[12]. The differential diagnosis for episodic vertigo/dizziness is broad and spans across various medical specialties. In fact, only a few patients would undergo thorough brain MRI, complete vestibular testing, ictal EEG, and professional molecular genetic testing[13]. Besides, if antiepileptic drugs (AEDs) are effective in treating vertigo/dizziness, the diagnosis of epileptic vertigo may also be a false-positive diagnosis. For instance, the use of AEDs is a successful strategy for the treatment of vestibular paroxysmia, vestibular migraine, and migraine[14,15]. Although the treatment response rate of AEDs in vertigo is as high as 90%, the diagnostic rate of epileptic vertigo is only 10%.

We should be alert in diagnosing cases of epilepsy presenting with vertigo as a standalone symptom since this condition is often underdiagnosed. This case demonstrates that to diagnose this condition, there is a need for a detailed history, a comprehensive physical examination, and a wide range of ancillary examinations and differential diagnoses, including those related to neurology, otolaryngology, internal medicine, and psychiatry. Multidisciplinary collaboration has the potential to cover areas that are underemphasized in a single professional context.

4. Conclusion

This patient initially experienced simple vertigo, and her EEG revealed abnormal wave discharges at both sphenoidal electrodes, which were located at both temporal lobes. Her symptoms remarkedly improved with regular antiepileptic therapy. This case may provide some reference for clinical diagnosis and treatment. However, there are some limitations observed in this case. Video EEG test was not done for the patient, neither was flash stimulation or sleep induction used during her EEG examination. Since this case report only covers one case, there may be certain contingencies; hence, more practical cases and theoretical studies are needed to make up for the deficiency. In the future, we must pay close attention to the history of such epileptic patients.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Data curation: Yanqiang Wang
Formal analysis: Xiaojun Zhang
Writing – original draft: Ning Wu
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Ethics approval and consent to participate

This study was approved by the Local Ethics Committee of the Affiliated Hospital of Weifang Medical University. The research ethics approval number was wyfy-2022-ky-179. The written informed consent for this study was obtained from the patient.

Consent for publication

The written informed consent and permission to publish the patient's data and/or images were obtained from the patient. We did our best to mask or conceal any identifying information of the patient that appears in writing or within photograph.

Availability of data

Not applicable.

References

1. Kim DW, Sunwoo JS, Lee SK, 2016, Incidence and localizing value of vertigo and dizziness in patients with epilepsy: Video-EEG monitoring study. *Epilepsy Res*, 126: 102–105.

   https://doi.org/10.1016/j.eplepsyres.2016.07.002

2. Hewett R, Bartolomei F, 2013, Epilepsy and the cortical vestibular system: tales of dizziness and recent concepts. *Front Integr Neurosci*, 7: 73.

   https://doi.org/10.3389/fnint.2013.00073

3. Tarnutzer AA, Lee SH, Robinson KA, Kaplan PW, *et al*., 2015, Clinical and electrographic findings in epileptic vertigo and dizziness: A systematic review. *Neurology*, 84: 1595–604.

   https://doi.org/10.1212/WNL.0000000000001474

4. Bisdorff AR, Staab JP, Newman-Toker DE, 2015, Overview of the international classification of vestibular disorders. *Neurol Clin*, 33: 541–550.

   https://doi.org/10.1016/j.ncl.2015.04.010

5. Strupp M, Lopez-Escamez JA, Kim JS, *et al*., 2016, Vestibular paroxysmia: Diagnostic criteria. *J Vestib Res*, 26: 409–415.

   https://doi.org/10.3233/VES-160589

6. Bladin PF, 1998, History of "epileptic vertigo": Its medical, social, and forensic problems. *Epilepsia*, 39: 442–447.

   https://doi.org/10.1111/j.1528-1157.1998.tb01398.x

7. Batu ED, Anlar B, Topçu M, *et al*., 2015, Vertigo in childhood: A retrospective series of 100 children. *Eur J Paediatr Neurol*, 19: 226–232.

   https://doi.org/10.1016/j.ejpn.2014.12.009

8. Jiang Y, Zhou X, 2020, Frontal lobe epilepsy manifesting as vertigo: A case report and literature review. *J Int Med Res*, 48: 300060520946166.

   https://doi.org/10.1177/0300060520946166

9. Surmeli R, Yalcin AD, Surmeli M, *et al*., 2020, Vertiginous epilepsy: Documentation of clinical and electrophysiological findings of nine patients. *Epileptic Disord*, 22: 775–781.

   https://doi.org/10.1684/epd.2020.1232

10. Hamed SA, Tohamy AM, Oseilly AM, 2017, Vestibular function in adults with epilepsy of unknown etiology. *Otol Neurotol*, 38: 1217–1224.

    https://doi.org/10.1097/MAO.0000000000001513

11. Kumar A, Sharma S, 2021, Complex Partial Seizure. In: StatPearls. Treasure Island: StatPearls Publishing.

12. Morano A, Carnì M, Casciato S, *et al*., 2017, Ictal EEG/fMRI study of vertiginous seizures. *Epilepsy Behav*, 68: 51–56.

    https://doi.org/10.1016/j.yebeh.2016.12.031

13. Malov AG, Kulesh AA, Vshivkov MI, 2019, Familial temporal lobe epilepsy 5 with vestibular seizures (a case report). *Zh Nevrol Psikhiatr Im S S Korsakova*, 119: 65–69.

    https://doi.org/10.17116/jnevro201911911265

14. Hewett R, Guye M, Gavaret M, *et al*., 2011, Benign temporo-parieto-occipital junction epilepsy with vestibular disturbance: An underrecognized form of epilepsy? *Epilepsy Behav*, 21: 412–416.

    https://doi.org/10.1016/j.yebeh.2011.05.017

15. Fotuhi M, Glaun B, Quan SY, *et al*., 2009, Vestibular migraine: A critical review of treatment trials. *J Neurol*, 256: 711–716.

    https://doi.org/10.1007/s00415-009-5050-5

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Before submitting for publication, please ensure that your paper and other supplementary files have been prepared and formatted in accordance with the guidelines below.

Submission structure, general style and format

Advanced Neurology requests that every new submission should be made and accompanied by 3 separate core files, namely manuscript, title page and back matter, and cover letter, whereas resubmission of revision file should be accompanied by 4 separate core files, namely manuscript, title page and back matter, cover letter, and response/rebuttal letter (collectively known as the revision file). Provision of supplementary files and/or confidential accessory files is optional or dependent on the nature of study and findings relevance. The table below briefly summarizes the type of files in a submission, their respective requirements and included items:

Type of file	File format	Requirements	Included items
(1) Manuscript	DOC or DOCX	- Use 1.5-spacing and format text in one column - Use page numbers and continuous line numbers - Font and size: Times New Roman, 12 - Insert tables and figures at the back of manuscript	- Manuscript title - Abstract (for original research article, review article and perspective article) - Keywords - Text - References - Tables (including caption and legend) - Figures (including caption and legend)
(2) Title page and back matter *	DOC or DOCX	- Use 1.5-spacing and format text in one column - Font and size: Times New Roman, 12	**<u>On the first page (title page):</u>** - Manuscript title - Authorship list (first and last names must be spelled out) - Author's affiliation, including department, institution, city, state, postal code, and country (indicated with superscript number) - Corresponding author information, including asterisk indication, mailing address and email - Indication of equally contributing authors (if any) with dagger symbol **<u>On the second page (back matter):</u>** - Acknowledgments - Funding - Conflict of interest (mandatory) - Author contributions (formatted as per CRediT) - Further disclosure about presentation of essential findings in conference(s) and/or upload of the paper to a preprint server
(3) Cover letter	DOC or DOCX	- Use 1.5-spacing and format text in one column - Font and size: Times New Roman, 12	- A brief explanation of what was previously known, the conceptual advancement with the findings and its significance to broad readership - A statement that "neither the manuscript nor any significant part of it is under consideration for publication elsewhere or has appeared elsewhere in a manner that could be construed as a prior or duplication of the same work" with author confirmation - If any, associated accession numbers or DOIs of the corresponding preprint version of the submission - [Optional] Name, affiliation and email address of up to 4 academically qualified (recommended) reviewers and/or name and affiliation of individuals who should be excluded from reviewing the submitted works on the grounds of conflict of interest
(4) Supplementary files		- Supplementary files should **not exceed 20 MB** in total (15MB individual file limit)	

- Supplementary tables or figures	DOC or DOCX	- Use 1.5-spacing and format text in one column - Use page numbers and continuous line numbers - Font and size: Times New Roman, 12 - Include both supplementary tables (editable) and figures (in JPEG, PNG or TIFF format) in the same file	- Supplementary tables - Supplementary figures
- Data set	XLS or XLSX	- All data should be neatly presented using consistent fonts	
- Videos	MP4	- If necessary, trim the video clip to focus only on essential parts, such as experimental procedures and findings or observation that can only be demonstrated using video(s) - Avoid unnecessary narrations that can be presented in written form	
(5) Confidential accessory files			
- Sample consent form (for human research only)	DOC, DOCX, PDF, JPEG, PNG or TIFF	- This is a sample, unsigned consent form that should bear the institution letterhead	
- Research ethics proof (for human and/or animal research only)	DOC, DOCX, PDF, JPEG, PNG or TIFF	- Ideally, this document should contain the essential research ethics information, such as ethics approval identifiers and the name of Institutional Ethics Review Board or Institutional Review Board - The research described in **original research article** should match the proposed research or significantly fit within the framework of the specification stipulated in the research ethics proof	
(6) Response/rebuttal letter (only applicable to revisions)	DOC or DOCX	- All comments/feedback and responses/rebuttals must be clearly and concisely presented	- Reviewers' comments and feedback - Authors' responses

* Ideally, all information given in the title page and back matter file, except for the manuscript title, should remain the same from the point of submission to paper acceptance. Thus, authors are responsible to ensure that all information therein is accurate before making submission. Refer to **Authorship and Author Information** section on **About the Journal** for more information about *Advanced Neurology*'s authorship policy.

Submitting authors should refer to the relevant sections in the following for more detailed information.

Author metadata during submission

During the submission process, the submitting author must ensure that all particulars of author information, including full name, affiliation, and email address, are given in the author metadata column of the submission system. These particulars must exactly reflect those on the title page of the submission; this includes the author order of the authorship list. Provide authors' ORCID ID, if available.

Article types

(1) Original research article

An original research article is based on original, basic and applied research and/or analysis. This type of article aims to describe significant and novel research. Authors of original research articles must confirm that the essential findings presented have never been published or under consideration elsewhere.

This article type typically has at least 5 tables and/or figures in total, approximately 40 references, and 7,000 words (inclusive of Abstract and References).

(2) Review article

A review article provides scholarly survey as well as balanced summarization and highlights of recent developments in a research field or emerging/future trends. Authors should ensure that all perspectives from different works are linked in balanced and cohesive manner, taking into consideration different schools of thought.

This article type typically has at least 5 tables and/or figures in total, approximately 70 references, and 7,000 words (inclusive of Abstract and References).

(3) Perspective article

A perspective article contains the author's scholarly opinions on a particular subject area or topic. Unlike a review, a perspective article covers a more specific part of the field, aiming to provide new insights into the subject matter. However, these perspectives or opinions should be objective in line with the spirit of academia. A good perspective piece should stimulate further discussions and initiate novel experiments.

This article type typically has 5 tables and/or figures in total, approximately 70 references, and 7,000 words (inclusive of Abstract and References).

(4) Case report

A case report serves to communicate new observations or findings such as an unexpected or rare diagnosis, complication of a known disease, treatment outcome, or clinical course in the human patients, that have been learnt from the clinical practice. The case as described in a case report must involve an important area of health and the report should present a clear and clinically useful message.

This article type typically has 1-3 tables and/or figures in total, approximately 15 references, and 2,000 words (inclusive of Abstract and References). In *Advanced Neurology*, the abstract of a case report is unstructured and should be in the length of 100-150 words. The main text should contain 4 main sections: Background, Case presentation, Discussion, and Conclusion.

(5) Letters

This article type is a collection of unsolicited letters from the readers who wish to comment on specific articles published in *Advanced Neurology* or another field-related journal. Alternatively, a letter can be written on an unrelated topic of interest to the journal's readership.

Ideally, a letter should present an in-depth, scholarly re-analysis of a previously published article in *Advanced Neurology* or in another field-related journal, accompanied by the reader's constructive insights and comments. Letters containing new ideas, supporting data or data criticizing the indicated article may be subjected to peer review at editors' discretion. Authors should specify the intended recipient of the letters, i.e., Editor or specific author(s).

This article type typically has no more than 3 tables and/or figures in total, no more than 20 references, and 2,000 words (inclusive of References). No Abstract is required.

(6) Editorial

An editorial piece is a solicited, concise commentary that highlights prominent topics in particular issue. Alternatively, an editorial represents the official opinions of the editors on the journal or special issue.

An editorial piece should not exceed 1,000 words (inclusive of References). Typically, an Abstract is not required and only 1 figure or table is allowed.

(7) Special feature article

Special feature articles are invited papers highlighting the following aspects:

- hot topics in the field encompassed by *Advanced Neurology*;
- new guidelines, recommendations, etc. in the diagnosis and therapeutic management of neurological diseases; and
- policies that are of interest to the researchers and physicians of clinical neurology.

Discussion outcomes stemming from meeting reports can be published in special feature articles, as long as they are relevant to the above-mentioned aspects.

Special feature articles containing new ideas, data and/or perspectives may be subjected to peer review at editors' discretion.

This article type typically has 5 tables and/or figures in total, approximately 70 references, and 7,000 words (inclusive of Abstract and References).

(8) Erratum

Authors should contact the editors of *Advanced Neurology* (editor.an@accscience.com) if certain errors made by the journal are found. The editors will evaluate the impact of the errors and decide on the appropriate course of action. Any corrections to a paper are published at the sole discretion of the editors.

(9) Corrigendum

Authors should contact the editors of *Advanced Neurology* (editor.an@accscience.com) if certain errors made by the authors are found. The editors will evaluate the impact of the errors and decide on the appropriate course of action. Any corrections to a paper are published at the sole discretion of the editors.

Language

All submissions must be written entirely in good American English. Spelling and use of punctuations should conform to conventions in American English. Clarity and conciseness are critical requirements for publications; therefore, submissions that are not clearly written will be returned to authors. Authors must ensure that their manuscripts are submit-ready or publish-ready before making submission. The articles published in *Advanced Neurology* are in adherence with the publishable standards of academic and scientific writing.

Please note that utilizing a language editing service is not a guarantee of acceptance.

Letter capitalization

Use sentence case capitalization in all aspects of the submission. In sentence case, most major and minor words are lowercase (proper nouns, including name of organizations and name of guidelines, are an exception in that they are always capitalized for the first letter of each word, except for minor words, such as conjunctions and short prepositions). The first letter of the first word should always be uppercase.

Manuscript title

The title should capture the conceptual significance for a broad audience. The title should not be more than 50 words and should be able to give readers an overall view of the paper's significance. Titles should avoid using uncommon jargons, abbreviations and punctuation.

Abstract

The purpose of abstract is to provide sufficient information and capture essential findings and/or messages of the paper. For full-length article, the length of an abstract should be in the range of 200-300 words. The abstract should be **unstructured**. Abstract is needed in original research article, review article, perspective article, case report and special feature article.

Keywords

Each submission should be accompanied by 3-6 keywords. Avoid using abbreviations and acronyms in keywords, unless they are established standard keywords. Separate keywords with semi-colons (i.e, term1; term2; term3).

Abbreviations and acronyms

Define abbreviations and acronyms upon their first appearance, **separately**, in the abstract, main text, table legends, and figure captions and legends.

Sections in article

(1) Section headings

Section headings should be in boldface. Examples of section headings of different levels are shown in the following:

Primary level : **1. Heart disease**

Secondary level : **1.3. Risk factors for heart disease**

Tertiary level : **1.3.2. Hypertension**

Authors are suggested **NOT** to introduce further sub-sections after the tertiary level section (e.g., **1.3.2.1. High-salt diet**).

(2) Special sectioning requirements for an original research article

- The introduction should provide a background that gives a broad readership an overall outlook of the field and the research performed. It tackles a problem and states its important regarding with the significance of the study. Introduction can conclude with a brief statement of the aim of the work and a comment about whether that aim was achieved.

- **Materials and Methods.** This section provides the general experimental design and methodologies used. The aim is to provide enough detail to for other investigators to fully replicate the results. It is also required to facilitate better understanding of the results obtained. Protocols and procedures for new methods must be included in detail for the reproducibility of the experiments. Informed consent should be obtained from patients or parents before the experiments start and should be mentioned in this section. For human and/or research, research ethics information, such as ethics approval identifiers and the name of Institutional Ethics Review Board or Institutional Review Board, should be indicated in this section.

- This section focuses on the results and findings of the experiments performed. After (statistical) analysis, all results, including tables and figures, must be neatly presented. If necessary, this section can be sub-divided into multiple topical sub-sections.

- This section should provide the significance of the results and identify the impact of the research in a broader context. It should not be redundant or similar to the content of the results section.

- Use this section for interpretation only, and not to summarize information already presented in the text or abstract.

It is acceptable to merge both Results and Discussion as a single section.

Data and image processing

Post-acquisition processing of images, photos and figures should be kept minimum to ensure that the final figures accurately reflect the original data as it was captured and/or produced. Any alterations should be applied to the entire image. Any kind of alteration, including but not limited to brightness, contrast and color balance, has to be clearly stated in the figure legend and in Materials and Methods section. For simulated or model figures, the software used for production, editing, and/or processing should be mentioned. Presenting images in the same figure must be made apparent and should be explicitly indicated in the appropriate figure legends.

Data comparisons should only be made from comparative experiments (or data from the same experiment). Same piece of data or figure should not be used in multiple instances, unless the images/data describe different aspects of the same experiment (reasons must be stated, wherever appropriate, in this regard). If inappropriate image/data manipulation is identified after publication, the editors reserve the right to ask for the original data and, if that is not satisfactory, to issue a correction or retract the paper, as appropriate.

Unit of measurements

Use SI units.

Nomenclature of genus and species

Write in italics (e.g. *Escherichia coli*). The full genus and species names must be mentioned both in the manuscript title at the first appearance of an organism in an article. The abbreviation (e.g. *E. coli*) is allowed after first mention.

Nomenclature of genes, mutations, genotypes, and alleles

Write in italics. *Advanced Neurology* highly encourages the use the recommended names found in the gene nomenclature databases, for instance, **HUGO Gene Nomenclature Committee**.

Chemical compounds

Advanced Neurology requires authors to fulfill the requirements below while reporting and/or describing a chemical compound in articles:

Scenario	Requirements
Naming chemical compounds	Use either IUPAC conventions or common names such as cholesterol and cephalosporins
Reporting a new chemical compound	Provide the exact structure of the compound as well as sufficient data regarding the purity and identity of the compound
Reporting the use of a known chemical compound	Provide sufficient data regarding the source, purity and identity of the compound

Figures

Include all figures, including photographs, scanned images, graphs, charts and schematic diagrams, at the back of manuscript. Avoid unnecessary decorative effects (e.g., 3D graphs) and minimize image processing (e.g., changes in brightness and contrast applied uniformly for the entire figure should be avoided or minimized). All images should be set against white background.

All figures should be numbered (e.g., **Figure 1**, **Figure 2**) in boldface. Label all figures (e.g., axis, structures), and add caption (a brief title) and legend as a description of the illustration below each figure. Explain all symbols and abbreviations used. Each figure should have a brief title (also known as caption) that describes the entire figure without citing specific panels, followed by a legend, which is either the description of each panel or further description about the single image. Identify each panel with uppercase letters in parenthesis (e.g. (A), (B), (C), etc.) Figures must be cited in chronological manner in the text.

The preferred file formats for any separately submitted figure(s) are JPEG, PNG and TIFF. All figures should be of optimal resolution. Optimal resolutions preferred are 300 dots per inch (dpi) for RBG colored, 600 dpi for grayscale and 1,200 dpi for line art. Although there is no file-size limitation imposed, authors are highly encouraged to compress their figures to an ideal size without unduly affecting the legibility and resolution of figures.

If necessary, the editors may request author(s) to supply high-resolution and/or unprocessed images after submission or paper acceptance for pre-screening/review and production purposes, respectively.

Tables

Include all tables at the back of manuscript. Editable tables created using Microsoft Word are preferred. A table should be accompanied by a caption on top of it. Captions and legends (which are placed beneath table) should be concise. All tables should be numbered (e.g., **Table 1**, **Table 2**) in boldface. Explain all symbols and abbreviations used. Tables must be cited in chronological manner in the text.

Lists and math formulae

Lists and math formulae should be properly aligned and included within the main body of the manuscript. List them using Roman numerals in parenthesis (e.g. (I), (II), (III), (IV), etc.) Lists and math formulae must be cited in chronological manner in the text.

Lists and math formulae should be given in editable text and not as images. Use the solidus (/) for small fractional terms, e.g., X/Y. In principle, variables should be italicized.

Footnotes

Do not use footnotes.

In-text citations

Reference citations in the text should be numbered consecutively in superscript square brackets. Some examples:

- Negotiation research spans many disciplines[3,4].
- This result was later contradicted by Becker and Seligman[5].
- This effect has been widely studied[1–3,7].

Do not include citations in the Abstract.

Personal communications and unpublished works can only be used in the manuscript and are not to be placed in the References section. Authors are advised to limit such usage to the minimum. These should be made identifiable by stating the authors, year of personal communications or unpublished works, and the words “personal communication” or “unpublished” in parenthesis, e.g., (Smith J, 2000, unpublished).

References

This section is compulsory and should be placed at the end of all manuscripts. Do not use footnotes or endnotes as a substitute for a reference list. The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should be excluded from this section.

Authors being referenced are listed with their surname or last name followed by their initials. All references should be numbered (e.g., 1, 2, 3, and so on) and sequenced according to the order they appear as the in-text citations. References (especially journal article’s) should follow the general pattern: author(s), followed by year of publication, title of publication, abbreviated journal name in italics, volume number, issue number in parenthesis and lastly, page range or article ID. If the referred article has more than 3 authors, list only the first 3 authors and abbreviate the remaining authors as italicized “*et al*.” (meaning "and others"). Use of DOI is highly encouraged; include DOI, if available, after the page range or article ID. Examples of references for different types of publications are as follows:

(1) Journals

Journal article (print) with 1-3 authors:

Younger P, 2004, Using the internet to conduct a literature search. *Nurs Stand*, 19(6): 45–51.

Journal article (print) with more than 3 authors:

Gamelin FX, Baquet G, Berthoin S, *et al*., 2009, Effect of high intensity intermittent training on heart rate variability in prepubescent children. *Eur J Appl Physiol,* 105(1): 731–738.

Journal article (online) with 1-3 authors:

Jackson D, Firtko A, Edenborough M, 2007, Personal resilience as a strategy for surviving and thriving in the face of workplace adversity: A literature review. *J Adv Nurs*, 60(1): 1–9. http://doi.org/10.1111/j.1365-2648.2007.04412.x

Journal article (online) with more than 3 authors:

Hargreave M, Jensen A, Nielsen TSS, *et al*., 2015, Maternal use of fertility drugs and risk of cancer in children — A nationwide population-based cohort study in Denmark. *Int J Cancer*, 136(8): 1931–1939. http://doi.org/10.1002/ijc.29235

(2) Books

Book with 1-3 authors:

Schneider Z, Whitehead D, Elliott D, 2007, *Nursing and Midwifery Research: Methods and Appraisal for Evidence-based Practice*, 3rd edn, Elsevier Australia, Marrickville, NSW, 112–130.

Book with more than 3 authors

Davis M, Charles L, Curry MJ, *et al*., 2003, *Challenging Spatial Norms*, Routledge, London, 12–30.

Chapter or article in book

Knowles MS, (eds) 1986, Independent study, in *Using Learning Contracts*, Jossey-Bass, San Francisco, 89–96.

(3) Preprints

Preprint article with 1-3 authors:

Ulgen A, Gurkut O, Li W, 2019, Potential Predictive Factors for Breast Cancer Subtypes from a North Cyprus Cohort Analysis. *medRxiv*. https://doi.org/10.1101/19010181

Preprint article with more than 3 authors:

Wu S, Sun P, Li R, *et al*., 2020, Epidemiological Development of Novel Coronavirus Pneumonia in China and Its Forecast. *medRxiv*. https://doi.org/10.1101/2020.02.21.20026229

(4) Others

Proceedings of meetings and symposiums, conference papers:

Chang SS, Liaw L, Ruppenhofer J, (eds) 2000, *Proceedings of the twenty-fifth annual meeting of the Berkeley Linguistics Society, February 12–15, 1999: General session and parasession on loan word phenomena*. Berkeley Linguistics Society, Berkeley, 12–13.

Conference proceedings (from electronic database):

Wang T, Cook C, Derby B, 2009, Fabrication of a glucose biosensor by piezoelectric inkjet printing. *Proceedings of the Third International Conference on Sensor Technologies and Applications, 2009 (SENSORCOM-M'09)*, 82–85.

Online document with author names:

Este J, Warren C, Connor L, *et al*., 2008, Life in the clickstream: The future of journalism, Media Entertainment and Arts Alliance, viewed May 27, 2009, http://www.alliance.org.au/documents/ foj_report_final.pdf

Online document without author name:

Developing an argument, n.d., viewed March 30, 2009, http://web.princeton.edu/sites/writing/Writing_Center/WCWritingResources.htm

Thesis/Dissertation:

Gale L, 2000, The relationship between leadership and employee empowerment for successful total quality management, thesis, *Australasian Digital Thesis database*, University of Western Sydney, 110–130.

Standards:

Standards Australia Online, 2006, Glass in buildings: selection and installation, AS 1288-2006, amended January 31, 2008, *SAI Global database*, viewed May 19, 2009.

Government report:

National Commission of Audit, 1996, *Report to the Commonwealth Government*, Australian Government Publishing Service, Canberra.

Government report (online):

Department of Health and Ageing, 2008, Ageing and aged care in Australia, viewed November 10, 2008, http://www.health.gov.au/internet/main/publishing.nsf/Content/ageing

No author:

Guide to agricultural meteorological practices, 1981, 2nd ed, Secretariat of the World Meteorological Organization, Geneva, 10–20.

Note: When referencing an entry from a dictionary or an encyclopedia with no author there is no requirement to include the source in the reference list. In these cases, only cite the title and year of the source in-text. For an authored dictionary/encyclopedia, treat the source as an authored book.

Acknowledgments*

*This should be included in the title page and back matter file

This is an optional section where authors can acknowledge people and/or institutions that provided non-financial support and/or helped with the research and/or preparation of the manuscript. Examples of non-financial support include externally-supplied equipment/biological sources, writing assistance, administrative support, and contributions from non-authors.

Funding*

*This should be included in the title page and back matter file

Authors should declare all financial support and sources that were used to perform the research, analysis, and/or article publication. Financial supports are generally in the form of grants, royalties, consulting fees and others.

Conflict of interest*

*This should be included in the title page and back matter file

At the time of submission, authors must declare any (potential) conflicts or competing interests with any institutes, organizations or agencies that might influence the integrity of results or objective interpretation of their submitted works. For more information, see our **Conflict of Interest** section on **About the Journal**.

Author contributions*

*This should be included in the title page and back matter file

This section should be included in original research articles, review articles and case reports. In *Advanced Neurology*, we encourage authors to use **Contributor Roles Taxonomy (CRediT)** in describing each contributor's specific contribution to the scholarly output in the Author Contributions section.

Definitions of each contributor role as per CRediT are as follows:

Contributor role	Definition
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims.
Data curation	Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later re-use.
Formal analysis	Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.
Funding acquisition	Acquisition of the financial support for the project leading to this publication.
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection.
Methodology	Development or design of methodology; creation of models.
Project administration	Management and coordination responsibility for the research activity planning and execution.
Resources	Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools.
Software	Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.
Supervision	Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.

Validation	Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.
Visualization	Preparation, creation and/or presentation of the published work, specifically visualization/data presentation.
Writing – original draft	Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation).
Writing – review & editing	Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre- or post-publication stages.

Authors are advised to follow *Advanced Neurology*'s preferred style of writing the Author Contributions statement. See an example below:

Conceptualization: Ali Jackson, Helen Meyer

Investigation: Ali Jackson, Tom Lewis-Hans, Han Xiang

Formal analysis: Han Xiang

Writing – original draft: Ali Jackson

Writing – review & editing: Helen Meyer, Joshua O'Brien

Supplementary files

This section is optional and contains all materials and figures that are excluded from the manuscript. These materials, figures or additional information are relevant to the manuscript but remain non-essential to readers' understanding of the manuscript's main content. All supplementary information should be submitted as a separate file during submission.

Supplementary figures and tables should be submitted in a single, separate supplementary file, and must be numbered, for example, **Figure S1** and **Table S1**. All tables must be editable (preferably created from Microsoft Word). The acceptable formats of images and illustrations used in figures are JPEG, PNG and TIFF. Citations of these items must be appropriately referenced in the manuscript in chronological manner, for instance, "Additional information can be found in **Table S1**." Note the additional letter **S** helps distinguish the normal from supplementary items.

Data set file are usually prepared using Microsoft Excel (in XLS or XLSX format).

Videos (MP4 format), with a constituent maximum size of 15 MB, can be uploaded as part of the supplementary file.

Revision and response/rebuttal letter

If the editorial decision for a submission is major revision or minor revision, authors are advised to revise the manuscript (and possibly, the supplementary files) as per the review reports and resubmit the revision file, including the manuscript, title page and back matter, cover letter, and response/rebuttal letter, before the due date.

Revisions should be done on the latest version of the manuscript (or in some rare cases, edited manuscript provided by the editor) with the track change on. The revisions made should be described and/or clarified in the response/rebuttal letter; ideally, explanation about the revisions should be made clear with the help of page number and line number. If authors do not agree with reviewers' comments and suggestions, rebut their points with strong evidence and reasonable arguments.

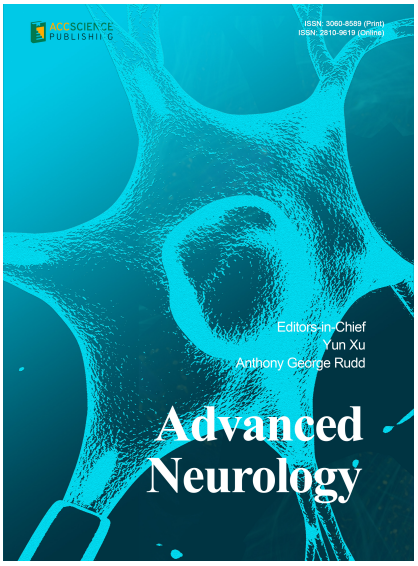
Advanced Neurology

Special Issue Alerts

Invitation for Special Issue Proposals

Organizing and editing for a Special Issue helps Guest Editors gain editorial experience and improve academic profile, in addition to being a part of organizing scientific communication of contemporary topics.

If you are published researcher and have an idea for a Special Issue, please write in via email to our Managing Editor (an.office@accscience.sg). Please provide your CV, professional profile page and a topic of interest in your email. Our colleague will guide you in the process of writing a Special Issue proposal.



Frequently Asked Questions

1. **Are Special Issue submissions processed in the same way how Regular Issue papers are being pre-screened and reviewed?**
   Yes, all full-length article submissions to a Special Issue will go through the same editorial and peer-review process. The distinct difference here is that the Guest Editors will replace the usual editors and get involved in the making professional decisions on papers after peer review. Note that the specific roles of a Guest Editor could vary across Special Issues.

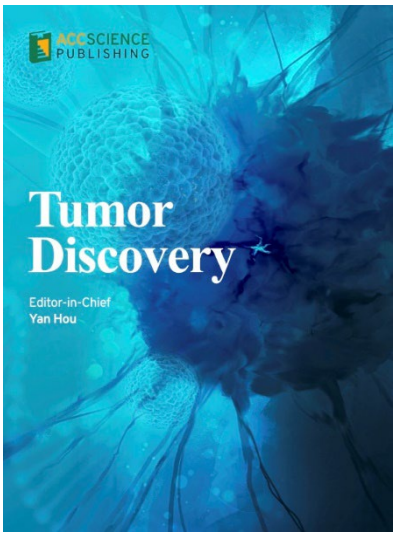
2. **How many Guest Editors are required to organize a Special Issue?**
   There is no fixed number; however, we suggest no more than 4 Guest Editors per Special Issue. More importantly, all Guest Editors should have excellent publication track records and demonstrated expertise in the topic(s) being proposed.

3. **Is the Special Issue governed by important deadlines?**
   Yes.

Benefits of Being A Guest Editor

- A chance to get involved in the conception and development of a specialty, contemporary topic that is of interest to the readers
- A chance to expand your professional network to the scholars and researchers who are similarly involved in the research of specialty topic
- A chance to hone your editorial skills
- A chance to gain first-hand experience of editing a thematic issue publication, which is a very valuable experience for those who aspire to edit their own journal in future
- A chance to improve your academic profile and help establish your academic influence within your discipline

Our Journals

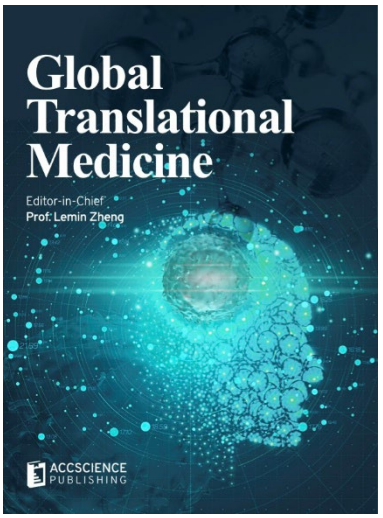


Tumor Discovery is a peer-reviewed and open-access journal that aims to present new cancer research with strong emphasis on fundamental and translational studies. *Tumor Discovery* covers topics, including but not limited to the following:

- Etiology and pathogenesis of cancer
- Mechanisms and molecular pathways underlying cancer initiation and progression
- Tumor metastasis
- Tumor evolution and heterogeneity
- Tumor microenvironment and tumor-host interactions
- Cancer genetics and genomics
- Cancer characterization using omics approaches
- Discovery and validation of cancer biomarker
- Discovery of new therapeutic targets
- New approaches of diagnostic and treatment modalities
- Statistical methods in cancer research

Global Translational Medicine is a quarterly journal that focuses on medicine, biological sciences, and biomaterials engineering. The goal of *Global Translational Medicine* is to provide a platform to researchers for showcasing their latest research works in translational medicine so as to advance the field towards the betterment of human health. Despite the advancement of omics and new technologies, the process of transforming these technologies and scientific research results into effective therapies and putting them into clinical use still has a long way to go. *Global Translational Medicine* provides a platform to fill the gaps in preclinical and inter-disciplinary research, to promote clinical translation of scientific research results, and to contribute to the conception of new and improved preventive measures as well as diagnostic and therapeutic techniques of diseases.

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