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Clinical Neuroscience Research

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The journal provides a platform for studies that explores the diagnosis, nature, causes, treatment, and public health aspects of neurological illnesses.

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Slack Channels as Key Regulators of Neuronal Excitability: Implications for Neural Function and the Link to Epilepsy Pathogenesis

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Abstract: The Slack channel encoded by the KCNT1 gene is a sodium-activated potassium channel. By regulating the flow of potassium ions, the Slack channel affects the membrane potential and discharge activity of neurons, thus participating in regulating neuronal excitability. Therefore, it plays a crucial role in maintaining the normal function of the nervous system. Consequently, abnormal Slack channel function is closely linked to various neurological diseases, such as epilepsy. Currently, quinidine-based medication therapy and neuroregulatory therapy are key components of the treatment of epilepsy resulting from Slack channel dysfunction. This article aims to outline the fundamental features of the Slack channel while providing a thorough analysis of the main distinctions and possible connections between Slick and Slack channels. Furthermore, this study focuses on the function of controlling the neuronal excitability of Slack channels while delving deeper into the potential correlation between Slack channels and epilepsy and their treatment strategies.

Keywords: Slack channel; Neuronal excitability; Epilepsy; Therapeutic strategies

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1. Introduction

The Slack channel, encoded by the KCNT1 gene, is a distinctive potassium(K^+) ion channel that is among the biggest subunits in the potassium channel family. The importance of it in the neurological system is of utmost importance, playing a vital part in neuronal activity. As an outwardly rectifying potassium channel, it helps K^+ ions flow out of the cell when the cell membrane is depolarized, which is crucial for regulating neuronal excitability and firing activity^[1-3]. This regulatory function is essential for ensuring normal signal transmission in the nervous system and helps maintain its balance and stability^[4-5].

Slack channel malfunctions can have a significant impact on the nervous system, potentially causing neuronal hyperexcitability or inhibition. This disruption can contribute to the development of various neurological diseases, including epilepsy^[4-8]. In epilepsy, dysfunction of Slack channels may result in neuronal hyperexcitability, triggering abnormal discharges and seizures^[9-11]. Therefore, conducting in-depth research on

the structure and function of Slack channels, as well as their relationship with neurological diseases, will not only help researchers obtain a deeper understanding of the basic working principles of the nervous system but may also provide new ideas and methods for the diagnosis and treatment of related diseases. This paper aims to provide a thorough overview of the most recent developments in the Slack channel, investigate its fundamental properties in more detail, and examine its crucial function in the nervous system. This article also addresses the Slack channel abnormalities linked to seizures to offer novel approaches to the treatment of epilepsy.

2. Basic characteristics of Slack channels

Research has shown that the sodium-activated potassium (K_{Na}) channel is encoded by two genes belonging to the SLO family: KCNT1 (also known as Slack, Slo2.2) and KCNT2 (Slick, Slo2.1), which play a crucial role in regulating neuronal excitability and discharge activities^[1, 3–4]. Regarding K_{Na} channels, the activation of the K_{Na} channel is closely related to the intracellular concentration of sodium (Na^+) ions. When neurons are stimulated, the accumulation of intracellular Na^+ ions can activate the Slack channel, triggering its opening and thereby regulating the flow of K^+ ions^[1, 4].

Slack channel is an important member of the K_{Na} channel family, and the molecular structure of the Slack channel is primarily composed of four major α -subunits, which exhibit a high degree of structural consistency^[11–13]. Each α -subunit contains a small intracellular amino-terminal domain, six transmembrane domains (S1–S6), and a large intracellular carboxy-terminal domain (**Figure 1**). Among these domains, S1 to S4 are primarily responsible for voltage sensing, with their charge distribution making them highly sensitive to changes in membrane potential^[14–15]. Additionally, the Slack channel possesses a longer C-terminal domain and contains regulatory domains for K^+ conductance (RCK) and an NAD^+ binding domain^[16–17]. The RCK domain regulates K^+ ion conduction through specific mechanisms, ensuring that ions can pass through the channel smoothly when needed, while the NAD^+ binding domain affects the activity of the channel through binding to NAD^+ ^[12]. Apart from these functional domains, the C-terminal domain of the Slack channel may also interact with various proteins such as FMRP, phosphatases, Phactr1 (a regulator of actin dynamics), and Cyfip1 (cytoplasmic FMR1-interacting protein 1), forming a complex protein network^[15, 18–19].

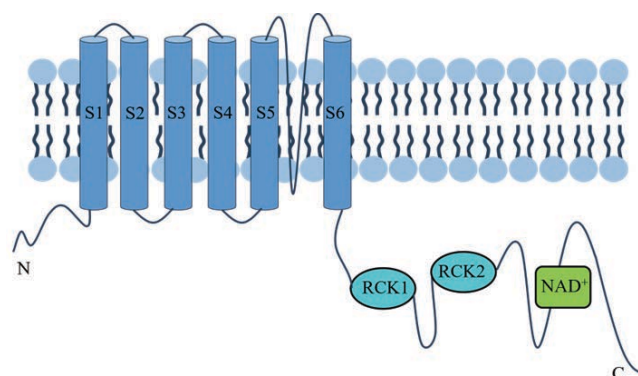


Figure 1. Schematic diagram of the Slack channel subunit

3. Comparisons between Slack and Slick

Similar to the Slack channel, the Slick channel encoded by the KCNT2 gene also plays a crucial role in the nervous system^[11, 20–21]. Their primary function is to regulate the flow of K^+ ions across the cell membrane in maintaining the transmembrane potential difference and regulating cell excitability^[3, 22–24]. Therefore, both Slack

and Slick channels have significant impacts on the electrophysiological properties of cells ^[11]. Furthermore, Slack and Slick channels are both K_{Na} channels, meaning that their open state is regulated by the concentration of Na^+ ions, which makes them essential in responding to external stimuli and maintaining cellular homeostasis ^[3, 24–26]. Additionally, Slack and Slick channels exhibit certain structural similarities. As potassium channels, they possess similar channel protein structures, including transmembrane regions, ion-selective filters, and regulatory domains ^[11]. These structural similarities contribute to their functional commonalities, such as their ability to selectively permeate K^+ ions and respond to external stimuli ^[3, 22, 27].

However, compared to the Slack channel, the Slick channel exhibits some differences. Firstly, despite their structural similarities, the Slick protein is slightly smaller than the Slack protein, with its N-terminal size being half of that of Slack (**Figure 2**). Furthermore, there is an ATP binding site in the C-terminal region of the Slick channel, and the application of adenosine triphosphate directly reduces Slick channel activity ^[23, 28]. In contrast, Slack channels do not exhibit such inhibitory effects. Additionally, Slick channels exhibit a more widespread distribution, existing not only in the central nervous system but also extensively in the peripheral nervous system, encompassing both sensory and motor neurons ^[25]. The distribution pattern of Slick channels in the brain nearly overlaps with that of Slack channels ^[29]. Apart from the brain, Slick channels are also expressed in organs such as the heart, skeletal muscles, lungs, and liver, further broadening their potential roles in physiological and pathological processes ^[25]. Additionally, the overall electrochemical properties of the Slick channel differ from those of the Slack channel. The opening of the Slack channel absolutely requires Na^+ ions, whereas the Slick channel exhibits a basic level of activity in the absence of Na^+ ions and has a higher half-maximal effective concentration (EC50) for Na^+ ions ^[3, 28]. Meanwhile, the activity of Slick channels can be enhanced by intracellular Cl^- ions concentration, and this effect is more pronounced in Slick channels compared to Slack channels ^[30]. Moreover, Slick and Slack channels exhibit distinct activation characteristics in response to cell depolarization. When a neuron is stimulated and generates an action potential, causing a rapid depolarization of the membrane potential, the Slick channel can rapidly open, allowing for a swift outflow of K^+ ions from the cell ^[28]. In contrast, the activation process of the Slack channel is relatively slow. Although it is also activated during cell depolarization, its opening rate is slower, resulting in a relatively slower outflow of K^+ ions ^[26, 31]. The rapid activation of the Slick channel enables it to quickly function during the generation of action potentials and restore the resting state of the membrane potential, ensuring rapid regulation of neuronal excitability ^[21, 28, 32]. The slow activation of the Slack channel, however, allows it to provide a sustained and stable outflow of K^+ ions during neuronal excitation, contributing to the maintenance of membrane potential stability and the prolongation of depolarization, thus enabling fine-tuned regulation of neuronal excitability ^[11, 33].

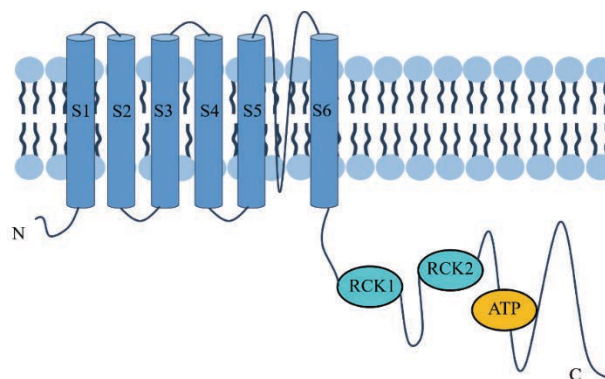


Figure 2. Schematic diagram of the Slick channel subunit

4. Slack channels regulate neuronal excitability

The Slack channel controls K^+ ion flow and membrane potential to modulate neuron excitability [12, 34–36]. When neurons are stimulated and generate action potentials, the influx of Na^+ ions causes membrane depolarization, which subsequently activates the Slack channels [33, 37]. Once activated, these channels mediate the outflow of K^+ ions, contributing to the repolarization of the membrane potential and returning it to the resting level [38]. This outflow of K^+ ions helps terminate the excitatory state of neurons, thus preventing neuronal hyper-excitability [38]. In addition, specific variants in Slack channels may lead to their enhanced function, increasing the rate of potassium influx or outflow, which in turn affects neuronal excitability. On the other hand, when neurons are in a resting state, Slack channels may maintain a relatively low level of openness, minimizing the outflow of K^+ ions and thus stabilizing the membrane potential [39–40]. Maintaining this balanced state is crucial for the normal functioning of neurons [15].

The cell membrane potential experiences slow afterhyperpolarization (sAHP), which is a state of prolonged, significant hyperpolarization, following the completion of an action potential [41]. This physiological phenomenon is a crucial component in the control of neuronal activity because it lowers the firing threshold of neurons, that is, the frequency at which neurons fire, by allowing the neuron to again cross the stimulus intensity threshold necessary for the release of action potentials [42]. After excitation, this inhibition aids neurons in swiftly returning to a resting state, preventing excessive discharge and energy consumption. Slack channels are essential to the sAHP process because they enable K^+ ions to move from inside the cell to the outside in a selective manner [41]. Since K^+ ions have a positive charge, their outflow causes the inner side of the cell membrane to become relatively more negatively charged, which exacerbates the hyperpolarized state of the cell. Thus, one of the key ways that the Slack channel controls neuronal excitability is by mediating K^+ ions outflow, which is necessary for the creation and maintenance of sAHP.

5. Slack channels in neurological diseases

5.1. Association of abnormal Slack channel function with epilepsy

Epilepsy, a chronic brain disease triggered by abnormal discharge of neurons in the brain, is closely related to abnormalities in neuronal excitability [43]. Studies have demonstrated that mutations in the gene encoding Slack channels are one of the important factors leading to certain focal epilepsies, commonly including epilepsy with migratory focal seizures (EIMFS) and autosomal dominant nocturnal frontal lobe epilepsy (ADNFE) [9, 32, 44–47].

Slack channels can modulate neuronal excitability and play a crucial role in the nervous system. Seizure-related mutation sites are found in various parts of Slack channels, such as the N-terminal domain, RCK1 domain, RCK2 domain, and C-terminal domain [32, 47]. These mutation sites can impact the functioning of Slack channels, including their opening, closing, and conductance properties, thus affecting the excitability of neurons. Gain-of-function (GOF) mutations in Slack channels lead to increased K^+ ions efflux, thereby shortening the action potential duration and increasing the excitability of neurons [40]. This excitability abnormality can disrupt the balance of the neuronal network and lead to excessive firing of the neuronal network, which in turn increases the risk of seizures [35, 48]. Additionally, Slack channels play a significant role in neural plasticity processes, and epileptic patients often exhibit changes in neural plasticity [4, 49]. By regulating neuronal excitability and synaptic transmission, Slack channels further influence the dynamic adjustment of neural network connections, thereby affecting the course of epileptic episodes and disease progression [50–51]. Consequently, the Slack channel plays a crucial role in the pathophysiology of epilepsy as a regulator of neuronal excitability. Its regular operation directly influences the frequency and mode of neuronal firing, which in turn influences the likelihood and duration of seizures [52–53].

5.2. Treatment strategies for abnormal Slack channel function

Being a crucial modulator of neuronal excitability, the Slack channel's malfunction is intimately linked to the development of numerous nervous system disorders, most notably epilepsy^[8]. Therefore, improving patients' quality of life greatly depends on the development of efficient treatment plans to address Slack channel dysfunction. Certain epileptic patients with Slack channel abnormalities may benefit from the use of traditional antiepileptic medications such as quinidine, phenytoin sodium, and carbamazepine^[40, 54]. Quinidine's clinical use must still be used with caution, though, as any potential side effects, such as cardiotoxicity, need to be properly monitored. It should be highlighted, furthermore, that not all patients with abnormalities of the Slack channel respond well to traditional antiepileptic medications, since these medications may not target Slack channels directly^[54]. Consequently, the development of novel antiepileptic medications that target Slack channels is required.

For some epilepsy patients who are refractory to treatment or who do not respond to medical intervention, surgery may be a viable option. Deep brain stimulation (DBS), focus excision for epilepsy, and other surgical techniques are available. Surgical treatment can significantly reduce or completely eradicate seizures by either directly removing the epileptic focus or by modifying the electrical activity of the neural network. For instance, pulsed magnetic fields are used in transcranial magnetic stimulation (TMS), a therapy that modifies the excitability of neurons in the cerebral cortex^[55]. In patients with Slack channel abnormalities, TMS may reduce the number of seizures or improve other neurological symptoms by regulating the balance of neuronal networks. This treatment method has the advantages of non-invasive and high safety, but the durability of efficacy and individual differences still need to be further studied. Furthermore, the pathogenic variants of the KCNT1 gene can be precisely repaired or replaced using gene editing technologies like CRISPR-Cas9, which will return potassium channels to their normal function^[56]. Although this treatment is drastic, more research and verification are still needed to fully understand its technical complexity and safety.

6. Conclusion and prospect

In conclusion, the dynamics of the nervous system depend on the Slack channel, also known as the K_{Na} channel, which is an essential component of the potassium ion channel. It achieves precise control over the excitability of neurons, the nervous system's ability to react rapidly to both internal and external stimuli and sustain normal function, by carefully regulating the flow of K^+ ions across their membranes. But if Slack channel function is compromised, the nervous system's capacity for adaptation and learning may be severely compromised, which could lead to the development of several nervous system disorders, including epilepsy. Furthermore, it has been demonstrated that modern anti-epileptic medications like quinidine and phenytoin sodium are crucial in the treatment of epilepsy brought on by Slack channel dysfunction. The frequency and intensity of seizures are effectively reduced and controlled by them by stabilizing and modifying the function of Slack channels.

Despite the established significance of Slack channels in the modulation of neural systems, the precise biochemical pathways and regulatory structures behind them remain incompletely understood. More research is required to understand how Slack channels interact with other ion channels, receptors, and signaling molecules. Moreover, further research is still needed to determine how Slack channel expression and function are impacted by mutations in the KCNT1 gene, and how these modifications contribute to the development of epilepsy. Currently, the primary treatment for epilepsy related to KCNT1 is medication therapy. But there are drawbacks as well, like patchy effectiveness and glaring negative effects. Therefore, to maximize treatment outcomes and enhance patient quality of life, future research should concentrate on the development of antiepileptic medications with high specificity and low side effects. These investigations will contribute to the growing

understanding of the function of Slack channels in neurological disorders and offer fresh perspectives on the identification and management of associated illnesses.

Disclosure statement

The authors declare no conflict of interest.

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Ameliorative Effect of *Hedysarum polybotrys* Polysaccharide on Neural Tissue Fibrosis in Diabetic Peripheral Neuropathy Mice and its Mechanisms

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Abstract: *Objective:* This study aimed to investigate the role of *Hedysarum polybotrys* polysaccharide (HPS) in ameliorating neural tissue fibrosis in diabetic peripheral neuropathy (DPN) mice. *Methods:* Fifty DPN mice were selected and randomly divided into five groups ($n = 10$), which were the model group, positive control group (receiving only 4 mg/(kg-d) of α -lipoic acid), high-dose HPS group (200 mg/(kg-d) of HPS was given per day), medium-dose HPS group (100 mg/(kg-d) of HPS was given per day), and low-dose HPS group (50 mg/(kg-d) of HPS given daily). In addition, non-diabetic C57BL/6 wild-type mice were selected as the normal group ($n = 10$). The expression levels of Keap1 and Nrf2 proteins and their mRNAs in the sciatic nerve tissues of mice in each group were analyzed by Western blot technique and real-time fluorescence quantitative PCR. *Results:* Compared with the normal group, the expression of Keap1 protein and mRNA was increased, while the expression of Nrf2 protein and mRNA was decreased in the sciatic nerve of mice in the model group ($P < 0.05$). Compared with the model group, Keap1 protein and mRNA expression decreased, while Nrf2 protein and mRNA expression increased in the control and high and medium dose HPS groups of mice ($P < 0.05$). *Conclusion:* HPS may inhibit fibrosis of neural tissue and ameliorate nerve injury in DPN mice by regulating the Keap1/Nrf2 signaling pathway. This effect was associated with enhanced antioxidant capacity, promotion of Nrf2 activation, and increased antioxidant gene expression by HPS. Therefore, HPS has a potential therapeutic value to ameliorate DPN-associated nerve injury.

Keywords: Hedysarum polybotrys polysaccharide; Diabetic peripheral neuropathy variant; Kelch1; Nrf2

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1. Introduction

Diabetic peripheral neuropathy (DPN) is a common complication that mainly affects the peripheral nerves leading

to sensory, motor, and autonomic dysfunction ^[1]. Symptoms of DPN include limb numbness, tingling, pain, and muscle weakness, which in severe cases may lead to foot ulcers and other complications. Effective management and treatment are essential to improve the quality of life of patients ^[2]. *Hedysarum polybotrys* polysaccharide (HPS), a natural active ingredient extracted from the traditional Chinese medicine *Radix Astragali*, is known for its multiple pharmacological activities, including antioxidant, anti-inflammatory, and immunomodulatory functions ^[3–4]. Recent studies have found that HPS has some efficacy in DPN treatment, and its mechanism may be related to its ability to enhance the body’s antioxidant capacity, thereby reducing the damage caused by oxidative stress ^[5]. This study aimed to investigate the ameliorative effect of HPS on neural tissue fibrosis of DPN in mice and its potential mechanism and to provide new ideas and theoretical basis for the prevention and treatment of DPN.

2. Materials and methods

2.1. Materials

2.1.1. Experimental animals

Six-week-old male SPF-grade NOD mice and C57BL/6 wild-type mice were selected (Suzhou Saiye Biotechnology Company). They were fed and watered freely in the SPF animal house with a light cycle of 12 hours, a room temperature of 22 ± 2°C, and a humidity of 50% ± 10%. The operation followed the regulations of the Animal Ethics Committee to ensure animal welfare.

2.1.2. Drugs and reagents

The drugs and reagents used are shown in **Table 1**.

Table 1. Sources of drugs and reagents

Drug name	Source
<i>Hedysarum polybotrys</i> polysaccharide (HPS)	Xinyang Mufan Biotechnology Co.
α-Lipoic acid capsule (300 mg/capsule)	Thermo Fisher Scientific
Keap1 monoclonal antibody	Thermo Fisher Scientific
Nrf2 monoclonal antibody	Thermo Fisher Scientific

2.1.3. Main instruments

The main instruments used in this study included SpectraMax Mini enzyme labeling analyzer (Shanghai Meigu Molecular Instrument Co., Ltd.), physiological recorder (Shanghai ShapeZoo Digital Technology Co., Ltd.), protein electrophoresis instrument, transmembrane instrument, and gel imaging analysis system, as well as real-time fluorescence quantitative PCR instrument (all purchased from Thermo Fisher Scientific).

2.2. Experimental methods

2.2.1. Model preparation

In this experiment, 6-week-old male NOD mice were used, which were divided into five groups of 10 mice each, including the model group, the positive control group, and the high-, medium- and low-dose HPS groups. Ten non-diabetic C57BL/6 wild-type mice were also selected as the normal group.

2.2.2. Drug administration method

All mice in the experimental groups were administered by gavage for 8 weeks. The specific dosing regimen

was as follows. Positive control group: α -lipoic acid was given at a dose of 4 mg per kg per time. HPS group: HPS was given to the high, medium, and low dose groups at doses of 200, 100, and 50 mg per kg per time, respectively. Model group and normal control group: an equal amount of saline was given at a dose of 5 mL per kg per time.

2.2.3. Sample collection

After 8 weeks of drug administration, blood samples were obtained from mice in each group by eyeball blood sampling. Subsequently, the mice were executed and dissected, and the right sciatic nerve tissue was removed. The collected sciatic nerve tissue was rinsed with pre-cooled saline to remove impurities. After absorbing the surface moisture, it was placed in liquid nitrogen for rapid freezing and finally transferred to a refrigerator at -70°C for storage for subsequent analysis.

2.2.4. Immunoblotting for Keap1 and Nrf2 protein expression

Protein extraction and quantification: about 50 mg was removed from the right sciatic nerve tissue preserved at minus 70°C , and total protein extraction was carried out according to the kit instructions. After the extraction was completed, the protein concentration was determined by the BCA method or other suitable methods.

Protein denaturation and electrophoresis: According to the measured protein concentration, each group of samples was denatured and the protein samples were separated using SDS polyacrylamide gel electrophoresis.

Membrane transfer and closure: membrane transfer is the transfer of separated Keap1 and Nrf2 proteins onto nitrocellulose membranes by electro-transfer method, which is usually carried out under electro-transfer conditions, such as 100 volts for 1 h. The transfer step is carried out at room temperature, using SDS polyacrylamide gel electrophoresis. The sealing step is performed by sealing the membrane using 5%–10% skimmed milk powder or BSA solution at room temperature, usually for 1 hour to reduce non-specific binding.

Antibody incubation: in the primary antibody incubation step, primary antibodies against Keap1 and Nrf2 are diluted, added to the membrane, and incubated overnight at 4 or for 1–2 hours at room temperature. Wash the membrane with PBS or TBST to remove unbound antibodies, add fluorescent labeling, incubate for 1 hour at room temperature, and wash the membrane again.

Develop and analyze: Develop or image the membrane (against the enzyme-labeled antibody) using an appropriate substrate (such as chemiluminescent substrate), or use a fluorescent imaging system. For data analysis, the relative expression levels of proteins are analyzed by the imaging software, which is usually normalized using an internal reference protein (such as β -actin or GAPDH) to ensure the reliability of the results.

2.2.5. Real-time fluorescence quantitative PCR to detect Keap1 and Nrf2 mRNA expression

Total RNA extraction and reverse transcription: 50 mg of right sciatic nerve tissue was collected and total RNA was extracted using TRIzol. RNA concentration and purity were determined by spectrophotometry, gel electrophoresis was performed to check the integrity, and 2 μg of RNA was transcribed into cDNA using a reverse transcription kit for PCR analysis.

Real-time fluorescent quantitative PCR: To construct the qPCR reaction system, a cDNA template, specific primers for Keap1 and Nrf2, fluorescent dye (e.g., SYBR Green), and a PCR reaction buffer were added. Transfer the reaction system to the qPCR instrument, set appropriate cycling conditions for PCR amplification, and record the fluorescence signal of each cycle to monitor the DNA amplification process.

Data analysis: at the end of amplification, the Ct values of Keap1 and Nrf2 were compared with those of the internal reference genes (such as GAPDH or β -actin) by analyzing the Ct values (the number of threshold cycles) to calculate the relative expression.

2.3. Statistical methods

Statistics were performed using SPSS 27.0, and the measurement data were expressed as mean \pm standard deviation (mean \pm SD), and *t*-tests were performed between groups. Statistical significance was indicated by $P < 0.05$.

3. Results

3.1. Comparison of Keap1 and Nrf2 protein expression in sciatic nerve tissues of mice in each group

Immunoblotting results showed that Keap1 protein was significantly higher and Nrf2 protein was significantly lower in the model group compared with the normal group ($P < 0.05$). Compared with the model group, Keap1 protein was significantly lower and Nrf2 protein was significantly higher in the control group and high and medium dose groups ($P < 0.05$). See Tab 2.

Table 2. Comparison of Keap1 and Nrf2 protein expression in each group (mean \pm SD)

Groups	Nrf2 protein	Keap1 protein
Normal group	0.90 \pm 0.03	0.61 \pm 0.08
Model group	0.50 \pm 0.06 ^a	1.13 \pm 0.07 ^a
Control group	0.87 \pm 0.04 ^A	0.67 \pm 0.06 ^A
Low dose group	0.59 \pm 0.08	0.97 \pm 0.03
Medium dose group	0.75 \pm 0.09 ^A	0.84 \pm 0.11 ^A
High dose group	0.84 \pm 0.08 ^A	0.87 \pm 0.12 ^A

Note: a indicates $P < 0.05$ compared with the normal group; A indicates $P < 0.05$ compared with the model group

3.2. Comparison of Keap1 and Nrf2 mRNA expression in sciatic nerve tissue of mice in each group

RT-qPCR results in **Table 3** showed that Keap1 mRNA was significantly higher and Nrf2 mRNA was significantly lower in the model group compared with the normal group ($P < 0.05$). Compared with the model group, Keap1 mRNA was significantly lower and Nrf2 mRNA was significantly higher in the control group and high and medium dose groups ($P < 0.05$)

Table 3. Comparison of Keap1 and Nrf2 mRNA expression in each group (mean \pm SD)

Groups	Nrf2 protein mRNA	Keap1 protein mRNA
Normal group	1.00 \pm 0.00	1.00 \pm 0.00
Model group	0.38 \pm 0.05 ^a	4.13 \pm 0.14 ^a
Control group	0.84 \pm 0.07 ^A	1.36 \pm 0.09 ^A
Low dose group	0.53 \pm 0.05	3.87 \pm 0.13
Medium dose group	0.72 \pm 0.08 ^A	2.44 \pm 0.10 ^A
High dose group	0.83 \pm 0.08 ^A	1.47 \pm 0.15 ^A

Note: a indicates $P < 0.05$ compared with the normal group; A indicates $P < 0.05$ compared with the model group

4. Discussion

HPS is an active ingredient extracted from the traditional Chinese medicine *Astragalus membranaceus*, which possesses a variety of pharmacological activities, including antioxidant and anti-inflammatory effects. In recent years, the potential role of HPS in the treatment of DPN has attracted widespread attention. Under normal physiological conditions, the binding of Nrf2 protein to Keap1 causes it to stay in the cytoplasm, at which time the activity of Nrf2 is inhibited and cannot perform its antioxidant function. When cells are subjected to oxidative stress or other deleterious stimuli, the conformation of Keap1 changes, and this change causes the binding of Nrf2 to Keap1 to be broken ^[6–7]. As a result, Nrf2 detaches from Keap1, which in turn translocates to the nucleus. Nrf2 enters the nucleus and binds to the antioxidant response element (ARE), which initiates the transcription of antioxidant genes ^[8]. Enhanced expression of these genes significantly enhances the antioxidant capacity of cells, enabling them to more effectively protect against oxidative stress and other potential damages. By regulating the activity of antioxidant genes, Nrf2 can enhance cellular defense mechanisms to ensure that cells maintain their normal functional and physiological states in response to environmental stress.

The present study showed that HPS significantly reduced Keap1 expression and elevated Nrf2 expression in mouse sciatic nerve. This suggests that HPS improves the occurrence and development of DPN by activating the Nrf2/Keap1 signaling pathway, enhancing antioxidant capacity, and alleviating oxidative stress damage. The regulation of the Nrf2-Keap1 pathway by HPS can be achieved through the following mechanisms. Promoting the entry of Nrf2 into the nucleus of the cell: HPS may enhance the expression of Nrf2 and Keap1 by decreasing the expression level of Keap1 or promoting its degradation, separation between Nrf2 and Keap1, thereby enhancing the biological activity of Nrf2. This mechanism helps Nrf2 to enter the nucleus more efficiently to fulfill its function of antioxidant action and regulation of cellular stress response and to enhance its transcriptional activity ^[9]. Enhancement of ARE activity: HPS can directly or indirectly enhance the activity of ARE through a variety of mechanisms, and this enhancement not only contributes to the activation of Nrf2 but also promotes the expression of antioxidant genes related to it, thus effectively enhancing the ability of cells to resist oxidative stress ^[10]. Thus, HPS can activate the Nrf2-Keap1 signaling pathway by regulating Keap1 and Nrf2 expression in the sciatic nerve, which enhances the antioxidant capacity of neuronal cells and has a significant anti-inflammatory effect, thus contributing to the amelioration of the various symptoms experienced by DPN patients. The findings of this study provide a new perspective and theoretical basis for the treatment of HPS in DPN, suggesting that HPS may be an effective intervention to help alleviate the discomfort associated with this type of neurological disease.

In summary, HPS has an ameliorative effect on mouse neural tissue fibrosis, and its mechanism may be related to the ability to up-regulate the expression of Nrf2, activate the Nrf2/Keap1 signaling pathway, improve the body's antioxidant capacity, and attenuate oxidative stress damage.

Disclosure statement

The authors declare no conflict of interest.

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Research on the Rehabilitation Effect of Repetitive Transcranial Magnetic Stimulation Combined with Cognitive Training on Children with Mental Retardation

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Abstract: This study investigated the rehabilitation effect of repetitive transcranial magnetic stimulation (rTMS) combined with cognitive training on children with mental retardation (MR). Through a randomized controlled trial design, 40 children aged 2–6 years with mental retardation were selected as study subjects and randomly divided into two groups: conventional treatment group and rTMS combined with cognitive training treatment group. The results showed that compared with the conventional treatment group, the rTMS combined with cognitive training treatment group exhibited more significant effects in improving children's cognitive function, social adaptability, and quality of life. This study not only enriched the theoretical basis of rehabilitation treatment for children with mental retardation but also provided strong evidence support for clinical practice.

Keywords: Repetitive transcranial magnetic stimulation (rTMS); Cognitive training; Mental retardation; Rehabilitation treatment; Children

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1. Introduction

Mental retardation is a common neurodevelopmental disorder in childhood, characterized by significantly lower intelligence levels than their peers, accompanied by inadequate social adaptability. This disorder not only severely affects children's daily lives and learning but also imposes a significant burden on their families and society. Rehabilitation treatment for children with mental retardation has relied heavily on special education, behavioral therapy, and medication, yet the efficacy of these methods is often limited, with varying degrees of side effects and limitations. Therefore, exploring more effective and safe rehabilitation treatment methods is crucial. With the continuous development of neuroscience and rehabilitation medicine, repetitive transcranial magnetic stimulation (rTMS), as a non-invasive brain stimulation technique, has gradually demonstrated potential applications in the rehabilitation treatment of children with mental retardation. rTMS promotes the connection and remodeling of

neurons by altering the excitability of the cerebral cortex, potentially improving children's cognitive function and social adaptability. Meanwhile, cognitive training, as a training method targeting specific cognitive domains, is also widely used in the rehabilitation treatment of children with mental retardation. However, current research on the effects of rTMS combined with cognitive training in the rehabilitation treatment of children with mental retardation is scarce. Therefore, this study explored the effect of rTMS combined with cognitive training on the rehabilitation treatment of children with mental retardation through a randomized controlled trial, providing a scientific basis for clinical practice ^[1-3].

2. Research subjects and methods

2.1. Research subjects

This study selected 40 children aged 2–6 years with mental retardation who visited the hospital and psychology department from January 2021 to December 2023 as research subjects. All children met the diagnostic criteria for mental retardation and excluded other diseases or factors that might affect the study results. The children were randomly divided into two groups using a random number table: conventional treatment group (20 cases) and rTMS combined with cognitive training treatment group (20 cases). The two groups were comparable in terms of age, gender, and severity of illness.

2.2. Research methods

2.2.1. rTMS treatment

rTMS treatment was performed using a repetitive transcranial magnetic stimulator, with treatment parameters adjusted according to the specific conditions and treatment needs of the children. In this study, rTMS treatment was administered three times a week, with each session lasting 20 minutes, for a total of 12 weeks. The treatment sites were primarily selected from areas of the cerebral cortex related to cognitive function, such as the frontal and temporal lobes ^[4].

2.2.2. Cognitive training

Cognitive training is tailored by professional rehabilitation therapists based on the children's cognitive level and developmental needs. The training content primarily encompasses attention training, memory training, thinking skills training, and social skills training. The training modalities incorporate game-based guidance, scenario simulation, emotional guidance, systematic training, and other diverse approaches, aiming to stimulate children's learning interests and motivation. The training frequency is set at five sessions per week, with each session lasting 25 minutes, spanning a total of 12 weeks ^[5].

2.2.3. Assessment tools

This study employs a range of assessment tools to evaluate the therapeutic effects on children. Key among them are the Gesell Developmental Schedules and the Wechsler Preschool and Primary Scale of Intelligence (WPPSI). These assessment tools provide a comprehensive and objective reflection of changes in children's cognitive functions and social adaptation abilities.

3. Research results

After 12 weeks of treatment and assessment, both groups of children demonstrated varying degrees of improvement in cognitive functions and social adaptation abilities. However, the improvement in the rTMS

combined with cognitive training group was the most notable. Specific findings are as follows.

3.1. Improvement in cognitive functions

In the rehabilitation treatment of children with mental retardation, the enhancement of cognitive functions serves as a crucial indicator of therapeutic efficacy. As an innovative non-invasive treatment method, repetitive transcranial magnetic stimulation (rTMS) combined with cognitive training exerts a positive influence on children's cognitive functions, particularly in speech comprehension and perceptual reasoning abilities, providing valuable practical experience and scientific evidence.

3.1.1. Enhanced speech comprehension

Speech comprehension is a cornerstone of children's cognitive development, and its significance cannot be overstated. It not only affects children's ability to engage in fluent language communication but also profoundly influences their cognition and understanding of the external world. This study used the Gesell Scale to observe progress in speech comprehension among children in the rTMS combined with cognitive training group. This progress manifests in multiple layers. Firstly, children's comprehension speed for words and sentences accelerates, enabling them to quickly grasp language information and interpret it well. Secondly, there is some improvement in their ability to comprehend complex contexts and infer implications beyond the literal meaning, indicating that they can not only directly understand the literal sense but also comprehend the speaker's intentions and emotions through non-verbal cues such as context, tone, and facial expressions. The enhancement in speech comprehension further triggers a chain reaction, fostering development in other cognitive domains. With a better grasp of language information, children's abilities in memory, thinking, imagination, and other aspects also improve correspondingly, forming a virtuous cycle ^[6].

3.1.2. Enhanced perceptual reasoning ability

Perceptual reasoning ability is a vital component of children's cognitive development, requiring individuals to process, analyze, and reason with perceived information using existing knowledge and experience. For children with mental retardation, the enhancement of this ability is particularly crucial as it directly relates to their ability to effectively tackle various challenges and problems in daily life.

In this study, children in the rTMS combined with cognitive training group also demonstrated improved scores in perceptual reasoning ability. They exhibited greater accuracy in processing visual, auditory, and other sensory information, enabling them to swiftly capture and process key information. Additionally, they showed progress in logical reasoning and problem-solving, with the most significantly improved children being able to apply learned knowledge and experience to effectively analyze and judge complex situations. This enhancement in perceptual reasoning ability has profound implications for the growth and development of these children. It not only helps them better adapt to school life and social environments but also lays a solid foundation for their future learning and work ^[7].

3.2. Improved social adaptation ability

Social adaptation ability, as a core element in children's growth process, plays a vital role in their overall development and future social integration. Following treatment, children's social adaptation ability also showed improvement, encompassing not only how they establish positive and healthy interpersonal relationships but also how they effectively adapt to complex and ever-changing social environments, as well as how they manage and regulate their emotions.

3.2.1. Enhanced social interaction skills

Social interaction is an integral part of children's social adaptation ability and serves as the cornerstone for their integration into society and the establishment of positive interpersonal relationships. For children with mental retardation, cognitive and emotional development delays often pose significant challenges in social interactions. In this study, children in the rTMS combined with cognitive training group demonstrated enhanced social interaction skills. These children showed higher levels of initiative and cooperation in their interactions with others. Before treatment, they passively awaited attention and guidance from others, but after treatment, they initiated conversations and engaged in activities more actively. This transformation boosted their confidence and comfort in social situations ^[8].

3.2.2. Improved self-care abilities

Self-care ability is a prerequisite for children's independent living and is one of the essential indicators of their social adaptation ability. In this study, children in the rTMS combined with cognitive training group made significant progress in self-care. Through systematic cognitive training, these children gradually acquired basic skills for daily activities such as dressing, eating, and personal hygiene. They gradually began to complete these tasks independently, reducing their reliance on others. This transformation not only improved their quality of life but also strengthened their self-confidence and self-esteem.

3.2.3. Improved emotional management skills

Emotional management is a crucial component of children's social adaptation ability and an essential safeguard for their mental health. Children with mental retardation often struggle to effectively manage their emotions due to cognitive limitations and immature emotional regulation mechanisms. In this study, children in the rTMS combined with cognitive training group showed improvement in emotional management. Through cognitive training, these children gradually learned to recognize and understand their emotions. They became aware of their emotional states and began to comprehend the impact these emotions have on themselves and others. They also acquired skills to express emotions appropriately and seek help when needed. Faced with difficulties and setbacks, they no longer resorted solely to crying or avoidance but instead sought solutions and actively reached out to others for support and assistance. This improvement in emotional management skills not only helps maintain a stable emotional state for the children but also enhances their psychological resilience and stress tolerance ^[9-10].

3.3. Improved quality of life

The improvement in quality of life serves as a crucial benchmark for evaluating treatment outcomes. In this study, comprehensive evaluations by parents and rehabilitation specialists revealed a notable enhancement in the quality of life among children in the rTMS combined with cognitive training group.

3.4. Enhanced self-confidence

The increase in self-confidence is a significant manifestation of improved quality of life for these children. Throughout the treatment process, as their cognitive functions and social adaptation abilities continued to improve, the children gradually recognized their progress and changes. This positive self-awareness not only bolstered their self-confidence and self-esteem but also ignited their motivation for further learning and exploration ^[11].

4. Discussion

4.1. Synergistic effects of rTMS and cognitive training

The results of this study indicate that rTMS combined with cognitive training exhibits significant synergistic effects in the rehabilitation of children with mental retardation. As a non-invasive brain stimulation technique, rTMS directly targets the cerebral cortex, promoting neuronal connections and remodeling, thereby improving the children's cognitive functions. Meanwhile, cognitive training, through targeted training activities, further consolidates and expands the therapeutic effects of rTMS, enabling comprehensive enhancement across multiple cognitive domains. This synergy not only enhances treatment efficiency but also strengthens the durability of the treatment outcomes ^[12].

4.2. Analysis of influencing factors

Age, as a significant marker of physiological development stages, exerts a notable impact on the response to disease treatment. Pediatric patients' brains and nervous systems are undergoing rapid development and are highly plastic. Therefore, younger children tend to exhibit stronger neuro-regenerative and adaptive abilities during treatment. For instance, in the treatment of neurological disorders such as cerebral palsy, autism spectrum disorder, or language development delay, early intervention can more effectively promote the remodeling of neural connections and accelerate functional recovery. Furthermore, young children's relatively weaker psychological defense mechanisms make them more receptive to new environments and treatment methods, which also contributes to improved treatment outcomes.

The severity of the condition is another crucial factor that cannot be overlooked when assessing treatment effects. Generally, children with milder conditions experience fewer functional impairments and have greater potential for recovery. These children often observe quicker symptomatic improvements, such as enhanced motor function, improved language abilities, or advanced social skills, during treatment. These positive feedbacks further strengthen their treatment confidence and motivation, creating a virtuous cycle. Conversely, children with more severe conditions may face more complex pathological mechanisms and broader functional impairments, resulting in longer treatment durations and potentially slower recovery rates. However, this does not imply that they cannot achieve significant improvements; they simply require more patient, sustained, and comprehensive treatment strategies.

Treatment adherence, or the degree to which patients and their families follow medical advice and actively participate in treatment activities, is one of the key factors determining treatment outcomes. Good treatment adherence ensures the complete implementation of the treatment plan, allowing children to receive adequate treatment doses and training time, thereby maximizing therapeutic effects. In contrast, poor adherence, such as frequent absences from treatment, irregular medication intake, or inadequate training intensity, can weaken treatment outcomes or even lead to treatment failure. Enhancing patients' and parents' awareness of treatment, strengthening doctor-patient communication, developing reasonable and feasible treatment plans, and conducting timely adherence assessments and adjustments are essential safeguards for ensuring treatment effectiveness.

4.3. Clinical application prospects

The potential of repetitive transcranial magnetic stimulation (rTMS) combined with cognitive training revealed in this study for the rehabilitation of children with mental retardation undoubtedly brings new hope to this special group. As a non-invasive treatment method, rTMS promotes neuroplasticity by modulating the excitability of the cerebral cortex, providing a scientific basis for improving children's cognitive function, attention, memory, and language abilities. Its non-invasive and painless characteristics make the treatment process safer and easier for children and their families to accept, effectively avoiding the potential side effects and long-term dependence

issues associated with traditional pharmacological treatments ^[13–14].

The integration of cognitive training further enhances the durability and comprehensiveness of treatment effects. Through systematic exercises, cognitive training specifically targets the enhancement of critical cognitive skills such as cognitive processing speed, working memory, and problem-solving abilities, complementing the physical stimulation of rTMS to collectively reshape and strengthen the brain's functional networks. This multimodal treatment model not only accelerates the rehabilitation process but also promotes the overall improvement of children's social adaptation abilities, laying a solid foundation for them to better integrate into society ^[15].

Looking ahead, the promotion and application of rTMS combined with cognitive training should become an essential direction in the field of rehabilitation medicine. Firstly, efforts should be intensified to promote public awareness of this treatment method and reduce misunderstandings and biases. Secondly, multi-center, large-sample randomized controlled trials should be conducted to verify its efficacy through more rigorous scientific methods and explore optimal combinations of treatment parameters. Concurrently, deepening research into the neurobiological mechanisms underlying the interplay between rTMS and cognitive training, revealing their pathways and targets, will provide a theoretical basis for personalized treatment planning. Lastly, as technology advances and costs gradually decrease, this treatment approach is expected to benefit more economically disadvantaged families, achieving equitable distribution of rehabilitation resources.

5. Conclusion

This study systematically explored the effects of repetitive transcranial magnetic stimulation (rTMS) combined with cognitive training on the rehabilitation of children with mental retardation through a randomized controlled trial design. The results demonstrated that rTMS combined with cognitive training can significantly improve children's cognitive function, social adaptation abilities, and quality of life. This method exhibits remarkable therapeutic effects and vast application prospects, warranting clinical promotion and application. Future research should further investigate its therapeutic mechanisms and optimize treatment protocols to better serve the rehabilitation of children with mental retardation.

Disclosure statement

The authors declare no conflict of interest.

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The Mediating Role of Emotion Regulation Strategies between Symptoms of Attention Deficit Hyperactivity Disorder and Anxiety-Depression Problems in Children

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Abstract: The purpose of this study was to investigate the mediating role of emotion regulation strategies of ADHD and anxiety-depression problems in children. Using a cross-sectional research design, this study collected a sample of children with ADHD and assessed their ADHD symptoms, anxiety-depression status, and emotion regulation strategies utilizing standardized tools. The data analysis results showed that emotion regulation strategies played a significant mediating role between ADHD symptoms and anxiety-depression problems, indicating that emotion regulation ability in children with ADHD may be an important factor affecting their anxiety and depression. This study not only provides a new perspective for understanding the emotional and behavioral problems of children with ADHD but also offers empirical evidence for developing targeted intervention measures. In the future, training in emotion regulation strategies is expected to become an important part of interventions for children with ADHD.

Keywords: Attention deficit hyperactivity disorder; Emotion regulation strategies; Anxiety-depression; Mediating role

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1. Introduction

1.1. Brief overview of the association between attention deficit hyperactivity disorder (ADHD) and anxiety-depression problems

Attention deficit hyperactivity disorder (ADHD) is a prevalent neurodevelopmental disorder in children, characterized primarily by symptoms of inattention, hyperactivity, and impulsive behavior ^[1]. This disorder has been recognized as a significant challenge for children's daily functioning and overall well-being. In recent years, research in the field of ADHD has gradually unveiled a complex and intricate relationship between ADHD and several comorbid conditions ^[2]. Based on a school-based study in Sweden, it is revealed that 87% of children with ADHD had one or more comorbid disorders.

Studies have demonstrated that children with ADHD are more likely to experience anxiety and depression.

Attention-deficit/hyperactivity disorder and mood disorder have been found to co-occur in 15% to 75% of cases in both epidemiologic and clinical samples of children and adolescents. The coexistence of anxiety and depression in children with ADHD poses significant challenges to their daily lives ^[3]. These comorbidities not only exacerbate the social difficulties that children with ADHD already face but may also lead to academic decline and a deterioration of their mental health status. The presence of anxiety and depression can further impair their ability to form and maintain peer relationships, leading to social isolation and loneliness. Moreover, the academic struggles that these children encounter can perpetuate a cycle of low self-esteem and increased stress, further exacerbating their mental health symptoms ^[4]. Given the substantial burden that ADHD and its comorbid anxiety and depression place on children's lives, it is particularly important to delve into the underlying mechanisms linking ADHD with anxiety and depression. Understanding the intricate relationships between these conditions can pave the way for more effective interventions and treatments that target the specific needs of children with ADHD and comorbid anxiety and depression. By unraveling the mechanisms underlying these associations, researchers and clinicians can develop tailored approaches to address the unique challenges faced by this population, ultimately improving their social, academic, and mental health outcomes.

1.2. Highlight the potential mediating role of emotion regulation strategies in the relationship between ADHD and anxiety-depression problems

Emotion regulation strategies refer to the cognitive and behavioral strategies that individuals adopt to achieve emotional adaptation when confronted with emotional stimuli ^[5]. These strategies play a crucial role in managing emotions and maintaining mental health. Considering that children with ADHD may have difficulties in emotion regulation, this study speculates that emotion regulation strategies could be a key mechanism connecting ADHD symptoms with anxiety and depression problems. Specifically, this study proposes that deficits in emotion regulation strategies among children with ADHD may contribute to the development of comorbid anxiety and depression. By exploring this potential mediating role, this study aims to gain a more comprehensive understanding of the emergence and development of emotional problems in children with ADHD. Understanding how emotion regulation strategies fit into the complex interplay between ADHD symptoms and anxiety-depression problems can inform the development of targeted interventions that address these emotion regulation difficulties, ultimately reducing the burden of emotional problems in this population.

1.3. Clarify the research objectives and hypotheses

The primary objective of this study is to verify the mediating role of emotion regulation strategies between ADHD symptoms and anxiety-depression problems in children with ADHD. This study aims to empirically investigate whether emotion regulation strategies serve as a bridge between ADHD symptoms and the emergence of anxiety and depression in this population ^[6].

The hypotheses are as follows. Emotion regulation strategies can effectively mediate the relationship between ADHD symptoms and anxiety-depression problems. This means that ADHD symptoms indirectly influence the emergence of anxiety and depression by affecting emotion regulation strategies. Deficits in emotion regulation strategies among children with ADHD contribute to the development and maintenance of comorbid anxiety and depression. Through empirical research, this study hopes to provide new ideas and directions for mental health interventions for children with ADHD. By focusing on emotion regulation strategies, this study aims to develop targeted interventions that can effectively address the emotional difficulties faced by children with ADHD, ultimately improving their mental health outcomes and overall quality of life ^[7].

2. Research methods

2.1. Description of research design, sample selection, and data sources

This study adopts a cross-sectional research design aimed at exploring the mediating role of emotion regulation strategies between ADHD symptoms and anxiety-depression problems in children with ADHD. The cross-sectional design allows for the collection of data from a population at a specific point in time, enabling the examination of relationships between variables without inferring causality. This approach is deemed appropriate for this study as it focuses on understanding the associations and potential mechanisms rather than establishing temporal precedence. In terms of sample selection, this study focuses on children with ADHD and considers their relevant characteristics such as age, gender, family background, and so on. To ensure sample diversity and representativeness, this study employs a multi-stage sampling strategy. Initially, this study collaborated with clinical settings, schools, and communities to identify potential participants. This approach allowed access to a wide range of children with ADHD from different contexts, enhancing the generalizability of the findings. To further ensure diversity, the study applied specific inclusion criteria, considering factors such as age range, gender distribution, and varying family backgrounds. This careful consideration aimed to capture the heterogeneity within the ADHD population and explore how emotion regulation strategies might differ across subgroups. Ultimately, the sample included 120 children who met the diagnostic criteria for ADHD. These children were assessed using standardized diagnostic tools and procedures to confirm their ADHD status. The sample size was determined based on feasibility and the need to have a sufficient number of participants to detect potential relationships and effects ^[8].

The data sources for this study were diverse, encompassing clinical records, parent and teacher reports, as well as direct assessments with the children themselves. Clinical records provided information on the diagnostic process, symptom severity, and any previous interventions. Parent and teacher reports offered insights into the children's behavior in different settings and helped validate the children's self-reports. Direct assessments included questionnaires and interviews designed to measure emotion regulation strategies, anxiety, and depression symptoms specifically tailored for children with ADHD. By including children of different ages, genders, and family backgrounds, this study aimed to obtain more comprehensive research results that could be generalized to a broader population of children with ADHD. This approach allowed exploring potential differences in emotion regulation strategies and their associations with anxiety and depression across various demographic and contextual factors ^[9].

2.2. Introduction of measurement tools and indicators

In terms of measurement tools and indicators, this study employed a variety of standardized instruments to assess ADHD symptoms, anxiety and depression issues, as well as emotion regulation strategies among children. Specifically, this study utilized ADHD rating scales, such as the SNAP-IV scale, to evaluate the severity of ADHD symptoms in children. This scale comprises multiple behavior items related to ADHD, which are rated by parents or teachers based on the child's actual performance ^[10]. Through this method, this study was able to obtain a comprehensive and objective assessment of the child's ADHD symptoms. To measure the child's anxiety and depression status, this study adopted the child anxiety and depression scale, such as the RCADS scale. This scale includes multiple emotion and behavior items related to anxiety and depression, which are filled out by the children themselves. This design aims to allow children to respond based on their true feelings, thereby more accurately reflecting their inner world.

Furthermore, this study used emotion regulation strategy questionnaires, such as the ERQ questionnaire, to assess the regulation strategies used by children when faced with emotional stimuli. This questionnaire includes multiple strategy items related to emotion regulation, which are also filled out by the children. Through this

questionnaire, this study was able to understand the specific practices and preferences of children in emotion regulation, and further analyze the relationship between these strategies and ADHD symptoms, as well as anxiety and depression issues.

2.3. Explanation of data analysis methods

In terms of data analysis, this study employed a systematic and rigorous approach to thoroughly examine the relationship between ADHD symptoms, anxiety-depression problems, and emotion regulation strategies in children. Initially, this study conducted descriptive statistical analysis to gain a comprehensive understanding of the basic characteristics of the sample and the distribution of various variables ^[11]. This step involved calculating descriptive statistics such as mean age, gender ratio, mean scores, and standard deviations of ADHD symptom scores, anxiety-depression scores, and emotion regulation strategy scores. By doing so, this study was able to establish a clear picture of the sample's demographic and clinical characteristics, which is essential for interpreting the subsequent findings. Subsequently, this study employed advanced mediation effect analysis methods to deeply explore the mediating role of emotion regulation strategies between ADHD symptoms and anxiety-depression problems. These methods allowed going beyond the surface-level associations and delve into the underlying mechanisms that connect these variables. Specifically, this study utilized regression analysis and structural equation modeling, which are powerful statistical techniques that enable the examination of complex relationships and the estimation of mediation effects ^[12].

Through these analysis methods, this study was able to more accurately reveal the potential mechanism of emotion regulation strategies in the relationship between ADHD symptoms and anxiety-depression problems ^[13]. This study constructed a structural equation model with ADHD symptoms as the independent variable, anxiety-depression problems as the dependent variable, and emotion regulation strategies as the mediating variable. This model allowed simultaneously estimating the direct and indirect effects of ADHD symptoms on anxiety-depression problems through emotion regulation strategies. By using regression analysis, this study tested the significance and magnitude of the mediation effect, providing valuable insights into the role of emotion regulation strategies in this relationship. Overall, the data analysis methods were designed to provide a comprehensive and nuanced understanding of the relationship between ADHD symptoms, anxiety-depression problems, and emotion regulation strategies in children. By employing descriptive statistical analysis, regression analysis, and structural equation modeling, this study was able to gain a deeper understanding of the complex interactions between these variables and the potential mechanisms underlying their relationships.

3. Research results

3.1. Presentation of basic characteristics and descriptive statistics of the sample

This study included a total of 120 children who met the diagnostic criteria for ADHD as samples. The demographic characteristics of the sample showed that the ratio of boys to girls was close to 1:1, with an average age of about 9 years (specific age range: 6–12 years), ensuring sample diversity and representativeness. In terms of ADHD symptoms, this study used a standardized ADHD rating scale to assess the sample. Descriptive statistics showed that the sample overall presented moderate to severe levels of ADHD symptoms, with a specific average score of 65 (standard deviation: 10 points), which was generally higher than the average score of the non-ADHD child population. For anxiety and depression issues, this study used the child anxiety and depression scale for measurement. The results indicated significant anxiety and depression symptoms in the ADHD child sample, with an average score of 58 (standard deviation: 8 points), significantly higher than the average score of the general child population, suggesting that ADHD children are more prone to emotional problems.

Regarding emotion regulation strategies, this study used the emotion regulation strategy questionnaire to assess the coping strategies of ADHD children when faced with emotional stimuli. Descriptive statistics showed that there were differences in the use of emotion regulation strategies among ADHD children, with some children tending to use adaptive regulation strategies, while others may use fewer or maladaptive strategies^[14].

3.2. Reporting the results of mediation effect analysis between ADHD symptoms and anxiety-depression problems through emotion regulation strategies

Through mediation effect analysis, this study further explored the mechanism of emotion regulation strategies between ADHD symptoms and anxiety-depression problems. The analysis results showed that emotion regulation strategies played a significant mediating role between the two. Specifically, ADHD symptoms indirectly affected the severity of anxiety-depression problems in children by influencing their use of emotion regulation strategies. The size of the mediation effect was moderate and reached statistical significance. Specifically, the proportion of the mediation effect value to the total effect value was 33%, indicating that emotion regulation strategies played an important role in the relationship between ADHD symptoms and anxiety-depression problems. Furthermore, the direction of the mediation effect was as expected, meaning that more severe ADHD symptoms were associated with a higher likelihood of children using maladaptive emotion regulation strategies, which further exacerbated their anxiety and depression problems.

4. Discussion and conclusion

The results of this study reveal the significant mediating role of emotion regulation strategies between ADHD symptoms and anxiety-depression problems in children, a finding that holds important theoretical and practical implications. This mediating role suggests that emotion regulation strategies play a crucial part in the relationship between ADHD symptoms and anxiety-depression problems. Targeting these strategies may be able to effectively address both the ADHD symptoms and the associated anxiety and depression in children. From an intervention perspective, the development of emotion regulation strategies is expected to be an effective way to improve anxiety and depression in children with ADHD. Teaching children effective emotion regulation skills may be able to alleviate their anxiety and depression symptoms, thereby enhancing their overall mental health. This approach offers a promising new direction for interventions aimed at improving the well-being of children with ADHD. However, it is important to note that this study also has certain limitations. Firstly, the sample size is relatively limited, which may affect the generalizability of the results. While the findings are promising, they may not be fully representative of the entire population of children with ADHD. Secondly, although the measurement tools used in this study are standardized, they still need further refinement to more accurately capture the complex relationship between children with ADHD and emotion regulation strategies. This is an important area for future research to focus on, as more precise measurement tools will allow for a deeper understanding of this relationship. Lastly, the cross-sectional study design limits the inference of causality. While this study has identified a mediating role for emotion regulation strategies, the direction of causality between ADHD symptoms, anxiety-depression problems, and emotion regulation strategies cannot be determined^[15].

To gain a deeper understanding of this field, future research can adopt a larger-scale longitudinal design to investigate the development of emotion regulation strategies over time and their impact on the symptoms of ADHD and anxiety-depression problems. This will help comprehend the long-term impact of emotion regulation strategies on the development of children with ADHD in a more comprehensive manner. Additionally, using more refined measurement tools to assess emotion regulation strategies will allow for a more accurate capture of the complex relationship between ADHD and emotion regulation, leading to a better understanding of this

phenomenon. Ultimately, this will enable the formulation of more effective intervention strategies for children with ADHD, addressing both their symptoms and associated anxiety and depression in a more targeted and effective manner.

Disclosure statement

The authors declare no conflict of interest.

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Effect of Blood Glucose Gap on Post-stroke Cognitive Impairment in Acute Ischemic Stroke Patients

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Abstract: *Objective:* To analyze the effect of the blood glucose gap on post-stroke cognitive impairment in acute ischemic stroke patients. *Methods:* 300 stroke patients admitted to the hospital between December 2021 and December 2022 were selected and divided into three groups according to the value of blood glucose gap: the group with no elevation of blood glucose gap ($n = 124$), the group with mild elevation of blood glucose gap ($n = 97$), and the group with elevated blood glucose gap ($n = 79$). The same treatment regimen was applied to these three groups and cognitive function was assessed using MMSE and MoCA at 3, 6, and 12 months after discharge. *Results:* The NIHSS and MoCA scores of the patients in the group with elevated blood glucose gap were significantly higher than those in the mildly elevated group and the non-elevated group at 3, 6, and 12 months after discharge, and the MMSE and MoCA scores of the patients in the group with mildly elevated blood glucose gap were significantly higher than those in the non-elevated group at 3, 6, and 12 months after discharge, and there were statistically significant differences between all the groups ($P < 0.05$). *Conclusion:* Patients with an elevated glycemic gap in acute ischemic stroke showed more pronounced cognitive impairment than those with no elevated glycemic deficit, and the severity of cognitive impairment increased with the degree of glycemic deficit.

Keywords: Glycemic gap; Acute ischemic stroke; Cognitive impairment

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1. Introduction

The significant impact of hyperglycemia on patient prognosis is widely recognized in a wide range of conditions, particularly in the prognostic assessment of conditions such as acute ischemic stroke, sepsis, and polytrauma, and its predictive value has been confirmed in several studies. However, the manifestation of this phenomenon in individuals does not necessarily correlate with a history of diabetes. It may arise from the occurrence of acute hyperglycemia in diabetic patients, or high baseline blood glucose due to acute physiological stress, or both of these factors may be present at the same time. This complexity adds to the difficulty of assessment to some extent. Recent studies have suggested that relying solely on hyperglycemia as a prognostic predictor may be subject to multiple biases, and have therefore introduced the concept of a “glycemic gap.” The glycemic gap is defined as a significant increase in blood glucose levels during stress^[1]. This concept provides a new perspective

to better understand and assess the impact of hyperglycemia on patient prognosis. Δ BG represents the difference between the blood glucose level on admission and the chronic blood glucose level (CBG), which is derived from the conversion of HbA1c. In the current diagnosis and prevention of post-stroke cognitive impairment (PSCI), biological indicators of Alzheimer's disease (AD), such as A-protein and tau protein, are usually introduced to support the diagnosis or prediction. However, the use of these indicators for accurate and early determination of PSCI is difficult and has low patient acceptance. Recent studies have found that the blood glucose gap can be applied by simply measuring glycated hemoglobin and blood glucose levels on admission and then deriving them by simple conversion, a method that is not only simple and convenient but also has obvious advantages in terms of wider application. However, literature is scarce on the use of the blood glucose gap in the analysis and prediction of PSCI, so this study aims to analyze and discuss this issue in depth.

2. General information and methods

2.1. General information

The target population of this study was 300 cases of acute ischemic stroke, 165 males and 135 females, who attended the hospital between December 2021 and December 2022. The age range of the patients was 41 to 70 years with a mean age of 52.34 ± 10.23 years. The time interval from onset to consultation ranged from 1 hour to 2 hours with a mean time of 1.45 ± 0.37 hours. All cases participating in the study met the diagnostic criteria listed in the Chinese Expert Consensus on Emergency Diagnosis and Treatment of Acute Ischemic Stroke in Chinese and Western Medicine.

2.2. Inclusion and exclusion criteria

Inclusion criteria: Patients with acute ischemic stroke within 7 days of onset were included according to the 2018 Chinese Acute Ischemic Stroke Diagnosis and Treatment Guidelines and etiologically typed according to TOAST typing.

Exclusion criteria: (1) those who were unwilling to attend; (2) those who had any mental abnormality, difficulty in verbal communication, and whose family members did not cooperate or were unable to follow up for other reasons; (3) those who were accompanied by tumors or severe liver or renal insufficiency; and (4) those who had acute infections.

2.3. Grouping method

At the time of admission, the relevant hematological indexes of acute ischemic stroke cases were collected and grouped based on the blood glucose gap. Among them, cases with a blood glucose gap of 0 were classified as the non-elevated group; cases with a blood glucose gap between 0 and 2.78 mmol/L were classified as the mildly elevated group; and cases with a blood glucose gap of more than 2.78 mmol/L were classified as the elevated group^[2].

2.4. Observation indicators

After the same treatment, the discharge time and follow-up time are set according to the actual situation of the cases.

2.4.1. NIHSS assessment

A rating scale was used to assess the degree of neurological deficit in acute ischemic stroke. The scale consists of several items and is mainly used to assess various aspects of neurological function such as the patient's level

of consciousness, language, motor ability, sensation, visual field, and coordination. The NIHSS was assessed at admission, 3 months, 6 months, and 12 months after discharge to regularly evaluate the treatment effect and thus to grasp the recovery progress of the cases. The NIHSS scores ranged from 0 to 42, with higher scores indicating more severe neurological damage. The specific grading criteria are: “0–1” indicates normal or nearly normal; “1–4” indicates mild stroke; “5–15” indicates moderate stroke; a score of “15–20” indicates a moderately severe stroke state; a score of “21–42” indicates a severe stroke state.

2.4.2. MoCA assessment

The MoCA scale is a cognitive assessment tool specifically designed to screen for mild cognitive impairment, covering multiple dimensions such as calculation, language, orientation, memory, attention and concentration, and executive function, with a full score of 30. When the score reaches 26, it is considered normal. MoCA was assessed at admission, 3 months, 6 months, and 12 months after discharge for the three groups of cases. Determination of postoperative cognitive disability (POCD) was achieved by the Newman method. The steps of this method were to compare the preoperative and postoperative MoCA test results with the standard deviation of the preoperative MoCA cognitive function score as a control. By calculating the difference between the preoperative and postoperative scores, the condition of POCD was considered to be fulfilled when the difference in more than one dimension exceeded one standard deviation.

2.5. Statistical methods

SPSS 16.0 statistical software was used to complete the analysis of statistical results. Measurement data obeying normal distribution were described by mean \pm SD, and independent samples *t*-test was used for comparison between groups; while skewed distribution measurement data were described by median (M) [Interval of Quartiles (IQR)], and Mann-Whitney *U*-test was used for comparison between groups. The counting data were expressed as percentages, and the χ^2 test was used for comparison between groups. Comparisons between groups were made using the χ^2 test; correlation analyses between the two groups were performed using Pearson or Spearman; and analyses were performed using dichotomous multifactorial and unordered multi-categorical multifactorial logistic regression.

3. Results

3.1. Comparison of general information of patients in three groups

In this study, patients were grouped according to different blood glucose gap values, of which 124 patients with normal Δ BG accounted for 41.33% of the total number; 97 patients with mildly elevated Δ BG accounted for 32.33% of the total number; and 79 patients with significantly elevated Δ BG accounted for 26.33% of the total number.

A comparison of the general data of the three groups showed that no significant differences were found in age, gender, and NIHSS score on admission ($P > 0.05$), except for a statistical difference in blood glucose gap values ($P < 0.05$). This indicates that the three groups of patients in this study were feasible and balanced in the later study. The specific data are shown in **Table 1**.

Table 1. Comparison of general data of the three groups of patients

Project/Groups	Group with non-elevated blood glucose gap (<i>n</i> = 124)	Group with mildly elevated blood glucose gap (<i>n</i> = 97)	Group with elevated blood glucose gap (<i>n</i> = 79)
Age (years)	52.16 ± 9.78	52.33 ± 9.89	52.43 ± 10.25
Sex (male, <i>n</i> %)	57 (34.55%)	53 (32.12%)	55 (33.33%)
Blood glucose gap (mmol/L)	-0.35 ± 0.09	2.45 ± 0.89	3.03 ± 1.12
NIHSS at admission (points)	26.9 ± 7.61	26.4 ± 8.01	26.7 ± 7.89
MoCA at admission (points)	28.37 ± 0.43	28.19 ± 0.36	28.32 ± 0.40
Time from onset to consultation (h)	1.40 ± 0.40	1.47 ± 0.42	1.43 ± 0.39

3.2. NIHSS assessment and comparison at admission, 3 months, 6 months, and 12 months after discharge

At the time of admission, there was no significant difference ($P > 0.05$) in the comparison of NIHSS assessment results among the three groups of patients, as shown in **Table 1**.

It was found that the NIHSS scores of patients in the group with elevated blood glucose gap were significantly higher than those in the mildly elevated and non-elevated groups at 3, 6, and 12 months after discharge. In addition, patients in the mildly elevated blood glucose gap group also had significantly higher NIHSS scores than those in the non-elevated group at 3, 6, and 12 months after discharge. There was a statistically significant difference between all groups ($P < 0.05$). The detailed data are shown in **Table 2**.

Table 2. Comparison of neurological function NIHSS scores before and after treatment in the three groups (points, mean ± SD)

Groups	On admission	After discharge		
		3 months after discharge	6 months after discharge	12 months after discharge
Group with non-elevated blood glucose gap (<i>n</i> = 124)	26.9 ± 7.61	14.2 ± 3.41	9.2 ± 3.23	6.2 ± 2.56
Group with mildly elevated blood glucose gap (<i>n</i> = 97)	26.4 ± 8.01	17.2 ± 4.10	11.2 ± 3.67	10.56 ± 3.76
Elevated blood glucose gap group (<i>n</i> = 79)	26.7 ± 7.89	25.4 ± 5.87	23.8 ± 4.89	22.4 ± 5.87
F/χ^2	0.321	3.511	4.245	5.232
P	0.786	0.043	0.039	0.033

3.3. Assessment and comparison of MoCA at admission, 3 months, 6 months, and 12 months post-discharge

At the time of admission, there was no significant difference ($P > 0.05$) in the comparison of MoCA assessment results among the three groups of patients, as shown in **Table 1**.

It was found that the MoCA scores of patients in the group with elevated blood glucose gap were significantly higher than those in the mildly elevated and non-elevated groups at 3, 6, and 12 months after discharge. Also, patients in the mildly elevated blood glucose gap group had significantly higher MoCA scores than the non-elevated group at 3, 6, and 12 months post-discharge. There was a statistically significant difference between all groups ($P < 0.05$). The detailed data are shown in **Table 3**.

Table 3. Comparison of neurological function MoCA scores before and after treatment in the three groups (points, mean \pm SD)

Groups	On admission	After discharge		
		3 months after discharge	6 months after discharge	12 months after discharge
Group with non-elevated blood glucose gap ($n = 124$)	28.37 \pm 0.43	16.46 \pm 0.68	12.65 \pm 0.57	8.46 \pm 0.48
Group with mildly elevated blood glucose gap ($n = 97$)	28.19 \pm 0.36	20.46 \pm 0.73	16.23 \pm 0.46	14.46 \pm 0.48
Elevated blood glucose gap group ($n = 79$)	28.32 \pm 0.40	26.46 \pm 0.53	23.46 \pm 0.40	20.46 \pm 0.48
F/χ^2	0.354	3.864	4.432	5.287
P	0.845	0.045	0.036	0.031

4. Conclusion

In recent years, the incidence and mortality rates of stroke have remained high in China and have attracted increasing attention because of its tendency to cause disability and recurrence^[3-6]. Once a stroke occurs, the lives and health of patients will be seriously affected. Among the many complications of stroke, post-stroke cognitive impairment is particularly prominent and extremely harmful. Therefore, early prevention and timely diagnosis and treatment become key factors. For post-stroke cognitive impairment (PSCI), glycemic parameters such as hyperglycemia, glycemic variability, glycated hemoglobin, and glycemic kinetics are considered potential risk factors^[7-9]. Studies have shown that hyperglycemia may impair cognitive function after stroke through several mechanisms. These mechanisms include increasing the production of reactive oxygen species, causing mitochondrial dysfunction, and triggering an inflammatory response, which ultimately leads to secondary neuronal damage, thus affecting the cognitive ability of patients. The studies of some scholars found that the indicator of mortality was significantly higher in the group with severely elevated blood glucose levels than in the group with mildly elevated levels and the normal group, and this difference was statistically significant^[10-12]. After logistic regression analysis, a key conclusion can be drawn: the new indicator of the blood glucose gap can effectively reflect changes in response to stressful blood glucose. Meanwhile, the blood glucose gap has also been shown to predict the prognosis of limb function in post-stroke patients, but its relationship with post-stroke cognitive impairment (PSCI) still lacks clear research results^[10]. Therefore, further exploration of the association between the blood glucose gap and PSCI will provide a richer basis for comprehensive management and treatment strategies for stroke patients.

The results of this study showed that patients in the mildly elevated glycemic gap group had significantly higher MMSE and MoCA scores than those in the non-elevated group at 3, 6, and 12 months after discharge, with statistically significant differences in all group comparisons ($P < 0.05$). These results suggest that an elevated blood glucose gap in acute ischemic stroke patients is significantly associated with increased cognitive impairment after stroke. There may be several reasons for this phenomenon: (1) Hyperglycemia and its variability may lead to increased inflammatory response, and this inflammation not only affects the microvascular function of the brain but may also lead to neuronal damage and death. (2) Elevated blood glucose gaps may be associated with oxidative stress, and the increase in reactive oxygen species may be toxic to cells, which in turn may affect the normal functioning of the nervous system. (3) Blood glucose fluctuations may affect the energy metabolism of the brain, resulting in nerve cells being unable to function properly with insufficient energy supply, thus exacerbating the manifestations of cognitive impairment. The occurrence of cognitive impairment worsened

as the degree of elevation of the blood glucose gap increased, suggesting that glycemic stability is critical to the cognitive health of stroke patients. Therefore, increased attention to glycemic management and effective measures to control glycemic fluctuations in the clinic can help improve the cognitive prognosis of patients and provide stronger support for stroke patients' recovery.

In summary, the blood glucose gap is defined as the difference between admission blood glucose and mean blood glucose, and as an emerging biomarker, it has important clinical significance in patients with acute ischemic stroke. Studies have shown that an elevated blood glucose gap is not only strongly associated with the incidence of post-stroke cognitive impairment, but may also be one of its predictors. A high blood glucose gap may reflect dramatic fluctuations in blood glucose levels, which can lead to intrinsic metabolic imbalance and oxidative stress in the body, further aggravating brain tissue damage and neuronal dysfunction. Therefore, by monitoring the blood glucose gap, clinicians can better assess the cognitive risk of patients and inform optimal post-stroke management.

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Advancements in the Study of Clinical Features and Molecular Functions in Heterogeneous TAF1-associated Clinical Phenotypes

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Abstract: *Purpose of review:* TATA-binding protein (TBP)-associated factor 1 (TAF1) encodes the largest component of the transcription factor IID (TFIID) complex, which binds to core promoters and serves as a scaffold for assembly of the RNA polymerase II transcription complex. Variants in TAF1 are associated with X-linked dystonia-parkinsonism (XDP) and X-linked syndromic mental retardation-33 (MRXS33). This review provides a concise summary of the genetic and clinicopathological features of TAF1 variants related to phenotype. *Recent findings:* XDP is an adult-onset X-linked progressive neurodegenerative disorder presenting dystonia and parkinsonism and caused by a SINE-VNTR-Alu (SVA)-type retrotransposon within *TAF1*. TAF1/MRXS33 intellectual disability syndrome is characterized by global developmental delay, intellectual disability, facial dysmorphism, generalized hypotonia, and neurological abnormalities due to the missense variants in TAF1. Various symptoms of TAF1 missense mutations may be related to mutations in different functional regions of the protein. The clinical manifestations of XDP and MRXS33, both caused by variants of TAF1, present prominent heterogeneity, which could be influenced by whether the TAF1 mutation is located in the coding region, the time when TAF1 expression decreases, and the effect on downstream gene expression. *Summary:* TAF1 is linked to many different phenotypes because of its variable regulation of coding and noncoding elements, which makes its mechanistic roles in disease challenging to interpret. However, it is important to note that strategies to correct TAF1 splicing could provide therapeutic benefits in different diseases.

Keywords: XDP; MRXS33; SVA; TAF1; Phenotypes

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1. Introduction

The transcription factor IID (TFIID) complex consists of TATA-binding protein (TBP) which includes 13 TBP-associated factors (TAFs). This complex promotes transcriptional initiation by recognizing promoter DNA and combining it with additional universal transcription factors for assembly into functional pre-initiation complexes^[1]. *TAF1*, encoding the largest component of TFIID (1893 amino acids), includes various complex functional domains that interact with other proteins or with DNA. Thus, it is reasonable that variants in *TAF1* could cause

diverse phenotypes. Indeed, variants in *TAF1* are associated with X-linked dystonia-parkinsonism (XDP) and X-linked syndromic intellectual developmental disorder-33 (MRXS33). XDP is an adult-onset X-linked progressive neurodegenerative disorder presenting dystonia and parkinsonism and caused by a SINE-VNTR-Alu (SVA)-type retrotransposon in intron 32 of *TAF1* ^[2]. It is suggested that the retrotransposon disrupts the splicing of *TAF1* mRNA and decreases the expression of *TAF1*. TAF1/MRXS33 intellectual disability syndrome is characterized by general developmental delay (GDD), intellectual disability (ID), facial deformities, generalized hypotonia, and variable neurological abnormalities due to missense variants in *TAF1*. Compared with the XDP, the publications linked to MRXS33 were limited and indicated considerable pleiotropy and clinical variability. The age of onset and clinical manifestations of XDP and MRXS33 are different, and the clinical symptoms are diverse. This paper aims to provide a brief explanation by summarizing the clinical symptoms of these two diseases; how the symptoms relate to different *TAF1* gene mutation patterns and the functions of different domains of the TAF1 protein; and recent advancements in research, both in vitro and in vivo, to understand these diseases.

2. Clinical characteristics of XDP and MRXS33 with TAF1 variants

XDP is an X-linked recessive disorder endemic to Panay Island in the Philippines, affecting 1 in 4000 males on the island ^[3]. One of the unique clinical features of XDP is the initial presentation of focal dystonia, which generalizes to multiple body regions over time. The dystonic phase of XDP predominates for the first 10–15 years after the patient develops symptoms before the Parkinsonian's symptoms become more predominant. Although the majority of individuals affected by XDP are male, XDP symptoms may also occur in female patients, producing focal, non-progressive dystonia; a staggering gait; and tremors ^[4]. Previous data indicated that the mean age of onset in men was 39 years, with a range of 12 to 64 years ^[5]. However, the mean age of onset in women was 52 years, with a range of 26 to 75 years ^[4]. Parkinsonian features tend to develop as the disease progresses, which may replace or accompany dystonic symptoms ^[3,5–6]. The non-motor features of XDP, as described in recently published studies, consist of cognitive impairment, alterations of mood (anxiety and depression), executive dysfunction, and impairments in abstract thinking and motor programming ^[7–9]. To date, over 500 males and 14 females have been identified as having XDP. The basal ganglia, from both clinical studies of patients and pathological studies of post-mortem tissue, have been an important study part of XDP research ^[5,10–12]. A review of magnetic resonance imaging (MRI) studies in patients with XDP showed that all cases with novel findings had hyperintense putamina rims, and 72% showed caudate head atrophy; however, putamina was detected in only 30% of the images, mostly during the later stage of parkinsonism ^[11]. Published studies of XDP genetics have confirmed one 2627 bp SVA retrotransposon insertion in intron 32 of *TAF1*, which exhibited a result on the splicing and expression of the TAF1 gene. In 2017, Bragg et al. found a full sequence of an XDP-specific SVA and identified one domain: the hexameric sequence, (CCCTCT)*n* with a variable number of repeats ^[13]. In a large sample of probands, the number of repeats of the hexamer ranged from 35 to 52 repeats and demonstrated a perfectly significant negative relationship with the onset age of disease in patients. This foundation was the first evidence confirming a direct correlation between sequence repeats in XDP patients and disease manifestation, thus indicating that the SVA played a significant role in disease pathogenesis. Westenberger et al. supported this notion and further suggested that the number of repeats of the hexamer had significant negative correlations with the onset age of XDP and with *TAF1* expression, and also positive correlations with the severity of disease and deficits of cognitive ^[14]. In 2021, a further study found that the number of repeats of the hexamer and the degree of repeat instability were higher in the basal ganglia and

cerebellum than in the blood ^[15].

Other than the special TAF1 variants associated with XDP, missense variants in *TAF1* have been shown to result in TAF1/MRXS33 intellectual disability syndrome (MIM# 300966). Indeed, variants of subunits of TFIID, such as *TBP*, *TAF2*, *TAF6*, and *TAF13*, have implied a possible correlation with neurodegenerative diseases and developmental delay. MRXS33 syndrome was initially reported by O’Rawe et al. in eleven independent families with nine distinct single nucleotide variants and two duplications including *TAF1* ^[16]. Patients displayed GDD, ID, characteristic facial deformities, generalized hypotonia, and variable neurologic abnormalities. It is worth noting that skewed X chromosome inactivation (XCI) was found in all affected and carrier females who tested, comprising asymptomatic heterozygous females in six independent families and one female proband ^[1]. Thus, it would be reasonable to propose that the XCI skewing test be performed to verify the pathogenic missense variants in *TAF1*. To date, sixty-one ID patients with missense variants in *TAF1* have been reported from forty-eight unrelated families (**Table 1**) ^[1,16–24]. Of these patients, 57 were male, and 4 were female (3 with de novo mutations, 1 unknown, and 2 with skewed XCI). TAF1 amino acid mutation sites were marked on the TAF1 protein (**Figure 1**), and most mutations were found in triple barrel-winged helix (WH)- α -helical (DUF3591) domain, which interacted with TAF7 and affected gene expression profiles during human development, and tandem bromodomains (BrDs), which are readers of acetyl-lysine residues at the center of histone acetylation signaling network ^[25–26]. The patients’ clinical information is summarized in **Table 1**, and this study classified the clinical manifestations into abnormal birth history; developmental delay; postnatal growth delay (including microcephaly); craniofacial malformation; cardiac malformation; musculoskeletal malformation (including abnormal sacral segmentation); ear, nose, mouth, eye, and throat problems; autism spectrum disorder (ASD); and epilepsy (**Table 1**). The paper also summarizes the clinical features of different *TAF1* missense variants located in different domains (**Table 1**). In addition to developmental delay, craniofacial malformation (41/61, 67.2%), musculoskeletal malformation (37/61, 60.7%), and postnatal growth delay (28/61, 45.9%) were the most common clinical manifestations of *TAF1* missense variants. Meanwhile, patients with amino acid mutation sites located in DUF3591, BrDs, and the non-functional region between the zinc knuckle motif and BrDs are more prone to clinical manifestations of ASD and epilepsy. However, further clinical information is needed to ascertain whether the developmental delay is more severe when the mutations are located in these parts, whether there are more specific clinical features, and why. A summary of brain MRI profiles demonstrates that hypoplasia of the corpus callosum is the most common neuroimaging feature in patients, followed by cerebellar atrophy and ventriculomegaly (**Table 1**).

Table 1. Summary of clinical Features of different TAF1 Missense variants located in different domains

Patient number	Male number	De Novo number	The domain of protein	Abnormal Birth History	Development Retardation	Postnatal Growth Retardation	Craniofacial Malformation	Cardiac Anomalies	Musculoskeletal Deformity	Ear, Nose, Mouth, Eyes, and Throat problem	ASD	Seizure
3	3	1	TAND	0	3	1	1	1	1	1	0	1
9	9	5		1	9	4	4	1	4	4	1	0
15	11	7	DUF3591	3	15	10	12	8	10	10	7	6
11	11	4		1	11	4	10	1	8	4	1	0
1	1	1	Zinc knuckle	0	1	0	0	1	0	0	0	0
5	5	1		1	5	3	4	3	5	5	4	1
17	17	5	BrDs	2	17	6	10	6	9	3	7	6

splicing pattern of *TAF1* mRNA in XDP and control iPSCs identified that similar levels of *TAF1* mRNA containing the microexon 34' are detected between XDP and control, as well as *TAF1* transcription is similar between XDP and control brains^[30]. Excision of SVA by genome editing of the XDP iPSC using CRISPR/Cas9-mediated gene editing rescued these defects, restored correct splicing, and normalized TAF1 transcript levels. Likewise, multiple studies have shown that the main genetic cause of XDP is decreased *TAF1* gene expression, whereas excision of SVA by gene editing increases *TAF1* expression^[31]. At present, there are three pathological mechanisms that may be involved in XDP: First, the XDP-specific nucleotide change (DSC3) is located in the exon after intron 32 of *TAF1*, which can affect a large number of expression of genes related to vesicular transport and dopamine function. In XDP patients, dopamine transporter imaging by single-photon emission computed tomography (SPECT) demonstrated decreased dopamine reuptake in presynaptic terminals in the bilateral putamen, and ultrasound changes in the substantia nigra were observed^[32–34]. Therefore, DSC3-mediated dopamine-centric gene dysfunction may be the molecular pathological mechanism of Parkinson-like symptoms in XDP patients. Second, changes in oxidative stress induced by *TAF1* may be involved in the occurrence of XDP. As early as 2016 and 2017, scholars have studied differential gene expression in fibroblasts from patients with XDP and fibroblasts from normal controls, showing the enrichment of genes related to the ability of cells to handle oxidative stress and the transduction of NF-κB signaling pathway-related inflammatory mediators are involved in the pathogenesis of XDP^[28, 35]. Third, increased glutamate receptor expression in XDP neurons results in neuronal excitotoxicity^[36]. In conclusion, there is abundant evidence from in vitro studies that the SVA insertion into intron 32 of *TAF1* is associated with the occurrence of XDP, but the specific mechanism remains to be further explored.

There are limited studies on the relationship between MRXS33 and *TAF1* missense mutation and the molecular pathological mechanism. O'Rawe et al. collected blood for RNA-seq studies in a family with two probands (p.Ile1337Thr)^[16]. That study found 213 genes to be differentially expressed between the affected male probands and their unaffected families. Transcription factor target enrichment analysis revealed a significant enrichment of genes regulated by E-box proteins (CANNTG promoter motifs), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis revealed an enrichment of genes involved in Parkinson's disease, Alzheimer's disease, Huntington's disease, and cardiac-muscle contraction. In a subsequent study, a novel variant in TAF1 (p.Ser1600Gly) resulting in MRXS33 showed that there was a different expression of neuronal ion channel genes between *TAF1*-deleted and control SH-SY5Y cells using a special PCR array covering 86 channel genes^[24]. Eight genes were confirmed, including *ASIC2*, *ASIC3*, *KCNJ14*, *CACNG4*, *KCNAB3*, *CACNA1G*, *HCN2*, and *KCNH2*, among which the latter three genes were downregulated, and *CACNA1G* was further evaluated, revealing that overexpression in TAF1 variant p.Ser1600Gly, remarkably down expression in *CACNA1G* and the protein level of CACNA1G (CaV3.1). The calcium imaging results were in line with the reduction in CaV3.1 protein. Both TAF1 depletion and the TAF1 variant p.Ser1600Gly could lead to defects in the length of dendrites and the number of interactions in neurons differentiated from SH-SY5Y cells, as well as cell proliferation by downregulation of *CCND1* in SH-SY5Y cells. This finding indicates that the TAF1 variant p.Ser1600Gly may cause clinical manifestations such as ID through loss of function.

In conclusion, *TAF1* mutations may cause loss of function in vitro studies, whether it is a *TAF1* missense variant in MRXS33 or an SVA insertion in intron 32 in XDP. According to RNA-seq and ChIP-seq, the difference in symptoms between the two may be related to neuronal development and ion channels, with the latter mutation being related to oxidative stress and glutamate receptor activation. However, it should be highlighted that the RNA-seq results are preliminary, and more experiments should be performed to confirm the expression of the key genes and molecular pathways.

4. Studies on in vivo levels of TAF1

To date, XDP studies on in vivo levels of *TAF1* have mainly focused on the brain tissue of patients; there are few XDP-related animal models. Autopsy studies of patients with XDP have shown that the loss of medium spiny neurons (MSNs) and astrocytes in the dorsal striatum correlates with the clinical presentation of XDP patients^[10, 37]. In XDP tissue, a marked loss of neuropeptide Y (NPY) interneuron staining was observed in the caudate nucleus and putamen, primarily exerting a slow modulation of its postsynaptic targets (MSNs)^[37]. Altered MSN modulation triggers hyperexcitability of cortical inputs, leading to neurotoxicity. Makino et al. used quantitative RT-PCR to find that the n*TAF1* isoform was significantly reduced in the XDP caudate nucleus as well as in the cortex and nucleus accumbens^[29]. These findings suggest that reduction of n*TAF1* may lead to neuronal loss in XDP brains. Similarly, Cirnaru et al. also found in animal experiments that targeted reduction of *ctaf1* and/or *ntaf1* in neonatal mice and rats resulted in a dyskinesia phenotype and a reduction in striatal cholinergic interneurons^[38]. Although the mouse and rat animal models established by Cirnaru et al. cannot represent the genetic animal models of XDP, they are the first animal models to show the specific roles of n*TAF1* and c*TAF1* in the nervous system.

Previous studies have shown that *TAF1* expression is necessary for early embryonic development in mice and *C. elegans*, so changes in *TAF1* expression may produce severe consequences^[39–41]. Therefore, few animal models have been established to investigate dysfunctional *TAF1* missense mutations associated with MRXS33. First, to explore the pathogenic mechanism involved in MRXS33 caused by a mutation in the *TAF1* gene, O’Rawe et al. designed a splice-blocking morpholino (MO) and CRISPR/Cas9 targeting to knockdown or disrupt *taf1*^[16]. The area of the optic tectum, occupying the majority of the space within the midbrain, was smaller in embryos injected with the *taf1* MO than in control embryos. This observation provided evidence for a functional link between a neuronal phenotype and *TAF1* mutations. In agreement with this observation, a recent study created the first complete knockout model of the *TAF1* orthologue in zebrafish by using CRISPR/Cas9 to investigate *taf1*’s role during embryogenesis, revealing that *taf1* knockout zebrafish embryos display lethal malformations implying embryonic lethality [1]. In conclusion, it can be inferred from the study that *TAF1* played an essential role in embryonic development and specifically in neurodevelopmental processes. Moreover, transcriptome analysis and Protein Analysis Through Evolutionary Relationships (PANTHER) of *taf1* zebrafish knockout suggest that *taf1* regulates genes that are important for neurodevelopmental processes^[1]. Because of the embryonic lethality of *TAF1* deletion, the animal model of MRXS33 which understands how mutations in *TAF1* contribute to neurological deficits failed. Thus, aside from the previous zebrafish model, a novel animal model in which the *TAF1* gene is deleted in rat pups was achieved by using CRISPR/Cas9 technology and somatic brain transgenesis mediated by lentiviral transduction^[42]. Either guide RNA (gRNA)-control or gRNA-*TAF1* lentiviral vectors were administered to rat pups by intracerebroventricular (ICV) injection on postnatal day 3, followed by a battery of behavioral tests on postnatal days 14 and 35^[42]. As predicted, the rat model replicated the clinical features of *TAF1* ID syndrome, with young rats showing motor deficits similar to those of juvenile humans. Histopathological analysis showed that *TAF1* deletion led to cerebellar and cortex abnormalities, where Purkinje cells were observed to be decreased in number as determined by Calbindin staining. The results suggested a possible cellular basis for the motor defects and morphological changes in the cerebellum and cerebral cortex, especially the loss of Purkinje cells. Further electrophysiological examination performed on Purkinje cells showed that the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) was significantly decreased below the control level with *TAF1* editing, and this change was associated with reduced CaV3.1 protein expression in the *TAF1*-edited animals^[43–44]. Then, it was found that treatment of SAK3, a T-type calcium channel enhancer, protected Purkinje and granule cells from apoptosis, restored sEPSCs in *TAF1*-edited Purkinje cells, and prevented the loss of cortical neurons and

GFAP-positive astrocytes by *TAF1* gene editing ^[45–46]. Overall, a *TAF1*-edited rat animal model suggests that *TAF1*-related dyskinesia may be associated with cerebellar Purkinje cell changes induced by presynaptic CaV3.1 deletion, but this animal model does not replicate all clinical manifestations of MRXS33, and new animal model and treatment strategies need to be developed.

In the currently developed animal model, knockdown of *Taf1* expression by gene editing causes a dyskinesia phenotype in postnatal rats and mice. However, posttranscriptional reduction of *c/nTaf1* expression does not cause cerebral or cerebellum morphological alterations, and knockdown of *Taf1* expression by CRISPR-targeted exon 1 contrasted with Purkinje cell loss and cortical abnormalities. Although the clinical symptoms presented in these two animal models do not fully represent XDP or MRXS33 but may serve as a hint: *TAF1* noncoding mutations cause XDP due to post-transcriptional modification, whereas *TAF1* missense mutations in *TAF1* cause MRXS33 due to loss of function.

5. Summary

Based on current knowledge, TAF1 is an important component of TFIID, regulating the expression of other genes that could account for the diseases associated with variants in *TAF1*. However, which downstream gene changes are caused by *TAF1* mutations and how the mutations relate to XDP and MRXS33 neuropathology are questions that remain to be fully investigated. In addition, the *TAF1* missense mutations found to be associated with MRXS33 were all located before exon 32, while the SVA insertion was in intron 32, and the decreased expression of *TAF1* in XDP may be related to post-transcriptional modification. The clinical manifestations related to *TAF1* mutation are related to the mutation located in the translated or untranslated region, but whether it is related to the location in different functional regions or before or after exon 32 needs further study. The downstream gene changes caused by different mutations and the molecular pathology of XDP or MRXS33 need to be fully studied. Only with this knowledge can treatments for *TAF1* mutation-related diseases be developed; importantly, it is expected that strategies to correct *TAF1* splicing could provide therapeutic benefits in a variety of diseases.

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Aquaporin-4 IgG Antibody Detection in Neuromyelitis Optic Spectrum Disorder

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Abstract: *Objective:* To develop a sensitive and reliable diagnostic approach for optic neuromyelitis (ONM), an overexpressing cell line capable of detecting aquaporin 4 (AQP4) antibodies was established. Subsequently, immunofluorescence was employed to detect AQP4 antibodies in serum and cerebrospinal fluid (CSF) samples from patients. Additionally, the clinical utility of AQP4 antibodies in the detection of ONM patients was analyzed. *Methods:* An aquaporin 4 expression plasmid was constructed and transfected into cell lines. Subsequently, indirect immunofluorescence was utilized to detect anti-AQP4 antibodies in serum and cerebrospinal fluid samples. *Results:* The indirect immunofluorescence detection system exhibited high sensitivity in the detection of 2241 clinical samples, with a positive rate of 16.8%. The positive proportion was in line with epidemiological data, and the positive situation was consistent with clinical symptoms. *Conclusion:* The engineered cell line exhibits superior detection performance for identifying antibodies present in serum and cerebrospinal fluid samples. The capability to detect anti-aquaporin 4 antibodies is pivotal for improving the efficiency of screening and diagnosing neuromyelitis optica, thereby possessing considerable potential for clinical application.

Keywords: Aquaporins; Autoantibodies; Ophthalmoneuromyelitis; Indirect immunofluorescence assay; Cell transfection

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1. Introduction

The aquaporin-4 (AQP4) protein is widely distributed in the central nervous system and primarily mediates the transport of water molecules in the brain tissue. In 2004, Lennon et al. confirmed the presence of self-antibody NMO-IgG in the serum of patients with neuromyelitis optica (NMO) ^[1]. This antibody can specifically bind to the microvasculature and other parts of the brain tissue, leading to neuromyelitis optica spectrum disorders (NMOSD). This specific antibody has been identified as an anti-AQP4 antibody. With further advancements in research, the detection of the anti-AQP4 antibody has begun to be applied in the auxiliary diagnosis of NMO. As noted in the 2021 Diagnostic and Treatment Guidelines for Neuromyelitis Optica Spectrum Disorders, AQP4-IgG is a highly specific diagnostic marker with a specificity of up to 90% and sensitivity of approximately 70%. Furthermore,

guidelines recommend the use of cell-based transfection immunofluorescence assays (CBAs) or flow cytometry techniques for serological examinations ^[2].

This study aimed to construct an AQP4 overexpressing cell line using the cell transfection technique mentioned in the diagnostic guidelines. Subsequently, an indirect immunofluorescence assay was performed to establish a detection protocol for AQP4-IgG antibodies in the samples. A total of 2,241 samples collected between January 2021 and December 2022 in the laboratory were tested for AQP4-IgG antibodies. The test results were then analyzed and summarized to assess the positivity rate of anti-AQP4 antibodies in central nervous system diseases, as well as the sex distribution among affected individuals. Additionally, this study discussed the clinical value of this antibody in the diagnosis and treatment of neuromyelitis optica spectrum disorder. To evaluate the effectiveness of the CBA method established in this study, a comparative experiment was conducted using commercial Elisa kits (RSR Limited: AQP4/96/2).

2. Materials and methods

2.1. Sample information

A total of 2,241 serum and cerebrospinal fluid samples were collected from January 2021 to December 2022. The samples were all patients with suspected NMO who were referred to the laboratory from neurology departments in various regions of China. The sex distribution among the samples was approximately 1:1.28 (male-to-female ratio). The mean age of the individuals included in the study was 50.44 ± 14.73 years. Additionally, the samples were carefully collected by nurses adhering to standard procedures and stored in designated tubes. The specimens were promptly transported to the research lab via cold chain logistics, ensuring their integrity for further analysis.

2.2. Gene synthesis

Retrieve the mRNA sequence for the AQP4-M23 (NM_001364286) gene from the NCBI database, followed by gene synthesis conducted by Nanjing Kingsley Company utilizing pcDNA3.1-eGFP plasmid vector.

2.3. Extraction and validation of the plasmid

The synthesized plasmid was introduced into *Escherichia coli* DH5 α cells through transformation, followed by cultivation in an aminobenzyl medium. Subsequently, positive monoclonal colonies were carefully selected for amplification and further culturing. After harvesting bacterial cultures, the recombinant expression vector was isolated using an endotoxin-free plasmid extraction kit (Tiangen).

The extracted plasmids were subjected to double digestion with NheI and EcoRV at 37°C for 15 min and 80°C for 20 min to inactivate the enzyme using the following digestion system. At the same time, the extracted plasmids were sent to Qingke Biological Company for sequencing verification.

Table 1. Digestion system

Digestion system	Dosage
pcDNA3.1(+)-C-eGFP-human AQP4	5 μ L
NheI	1 μ L
EcoRV	1 μ L
10 \times Buffer	2 μ L
ddH ₂ O	11 μ L

2.4. Cell culture and transfection

Hek293 (human embryonic kidney cells) cells were cultured and transfected with AQP4 gene expression plasmid according to the instructions using Lipofectamine3000 transfection reagent when 80%–90% confluence was reached. After 48 hours, the transfection effect was observed.

2.5. Fixation and permeabilization

Isolated cells with a transfection efficiency of up to 60%, and subjected them to structural fixation using 4% paraformaldehyde. Perform cell permeabilization using 0.2% Triton X-100, followed by blocking the cell slides with 3% BSA.

2.6. Validation using antibodies

First, the treated cell slides were incubated with a rabbit monoclonal AQP4-IgG antibody (Abcam catalog number: ab259318) at 35°C for 1 hour, followed by three washes with PBST. Next, goat anti-rabbit IgG labeled with TRITC (Abcam catalog number: ab6718) was added to the slides and incubated at 35°C for another hour, after which three washes with PBST were performed. Finally, the DAPI sealing reagent was applied and the cells were observed under a fluorescence microscope to verify transfection and protein expression.

2.7. Testing of samples

Before the test, the experimenters were unaware of the expected results of the samples. Serum samples (1:10) and cerebrospinal (1:1) were diluted, and the treated cell slides were incubated for 1 hour. The slides were washed three times with PBS, followed by incubation with Cy3-labeled anti-human IgG antibody (Jackson Immuno catalog number: 109-165-003) for 1 hour. The slides were washed three times with PBST again, DAPI sealing reagent was added, and the results were examined and recorded using a fluorescence microscope. The results were judged by two trained experimenters. In addition, for samples with very high fluorescence, further dilution can be performed.

2.8. Statistical analysis

Employing SPSS 19.0 statistical software for data processing and analysis, count data are presented as case numbers, age data as mean \pm standard deviation ($X \pm s$), and the Chi-square test was conducted for comparison between male and female groups, represented by (n (%)). A P -value less than 0.05 signifies a statistically significant difference.

2.9. Comparative experiment

Commercial ELISA kits (RSR Limited: AQP4/96/2) were procured for comparative studies, and the protocols were followed according to the manufacturer's instructions. Samples were randomly chosen and analyzed concurrently using both CBA and ELISA methods. It is worth noting that this commercially available kit can quantitatively detect AQP4 antibodies in samples, and the commercially available kits have already obtained medical device licenses within China, indicating their widespread utilization and acceptance within the domestic market. This ensures their reliability and effectiveness for various applications in the medical field.

3. Results

3.1. PCR validation results

Double-enzyme digestion and sequencing validation of the plasmids are presented in **Figure 1**. The digested

fragment larger than 6000 bp corresponds to the plasmid vector pDNA3.1(+)-C-eGFP (6176 bp), whereas the fragment smaller than 1000 bp represents the mRNA sequence of AQP4 (906 bp). Furthermore, the plasmid was subjected to sequencing analysis, and a comparison of the sequencing results with the theoretical sequence demonstrated a 100% alignment.

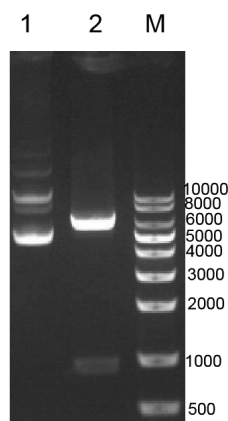


Figure 1. Enzyme digestion and electrophoresis. Lane 1: AQP4-pcDNA3.1(+)-C-eGFP: Recombinant plasmid; Lane 2: AQP4-pcDNA3.1(+)-C-eGFP: NheI and EcoRV Double Digestion; Lane M: 10KB Ladder

3.2. Results of the primary antibody reaction

Due to the presence of EGFP protein in the plasmid vector, transfected cells exhibited green fluorescence under the blue excitation wavelength range (420–485 nm) of a fluorescence microscope. Transfection efficiency can be analyzed based on the proportion of green fluorescent cells observed. Furthermore, incubation with the primary antibody and the addition of a red fluorescent-labeled secondary antibody resulted in red fluorescence in the green excitation wavelength range (460–550 nm), indicating successful expression of human aquaporin 4, as shown in **Figure 2**.

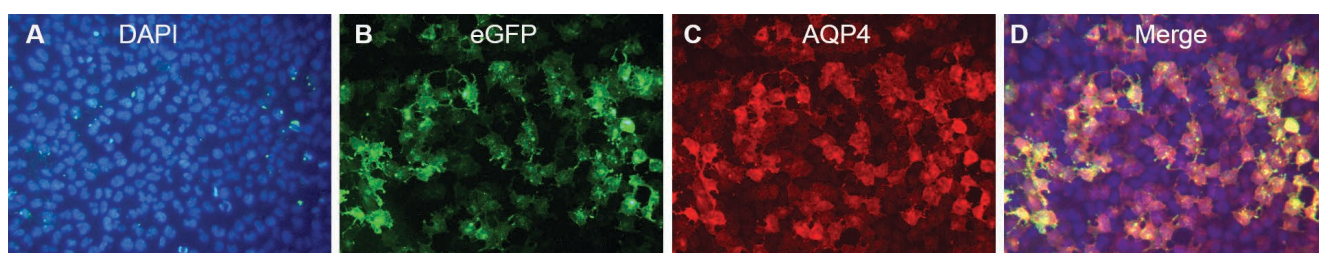


Figure 2. The expression of AQP4 protein in transfected cells was confirmed using an AQP4-igg antibody. A. DAPI staining of nuclei; B. eGFP was expressed in the cells; C. The antibodies in the sample bound to cellular antigens, followed by a secondary antibody labeled with fluorescence; D. A, B, and C were merged

3.3. Positive sample reaction results and their implications

Similarly, transfected cells exhibited green fluorescence in the blue excitation group (420–485nm). In the presence of an AQP4-IgG antibody-containing sample (positive sample), the antibody was bound to the AQP4 protein (antigen) expressed by the transfected cells, which was then bound by a Cy3-labeled anti-human IgG secondary antibody. Under the green excitation group (460–550nm) of the fluorescence microscope, red fluorescence was observed, which corresponded to the position of the green fluorescence. Conversely, in samples without AQP4-IgG antibodies (negative samples), only EGFP green fluorescence expressed by the cells was visible, devoid of any red fluorescence, as illustrated in **Figure 3**.

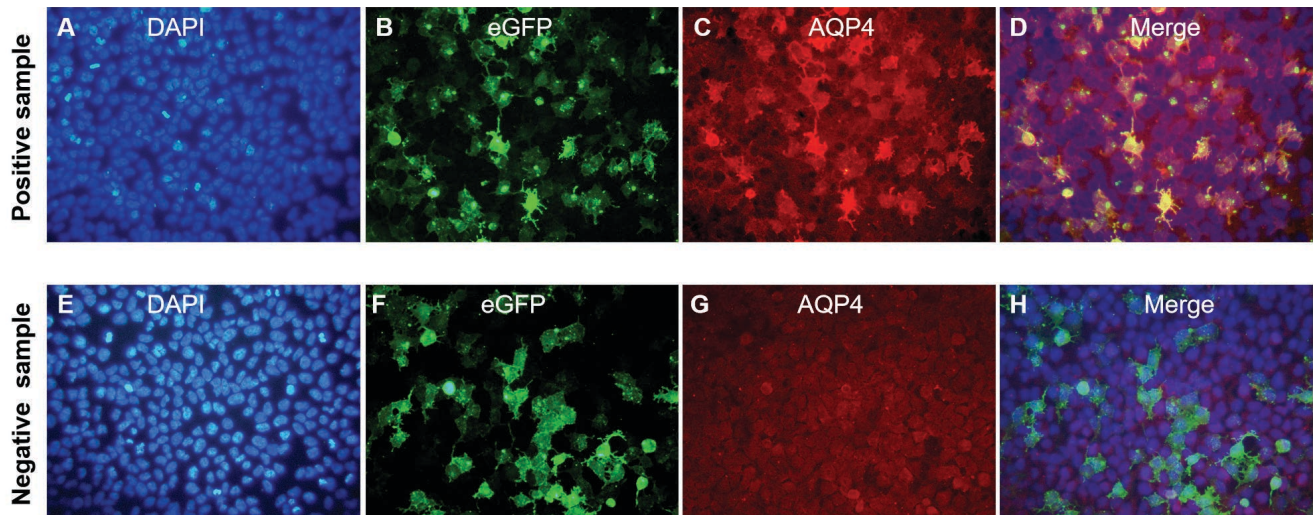


Figure 3. Fluorescence imaging of the positive and negative samples. A–D. The results of the positive samples under fluorescence microscope; A. DAPI staining of nuclei; B. eGFP tagged at the AQP4 C-terminus showing green fluorescence for the positive sample; C. The anti-AQP4 antibody in the positive samples binds to AQP4 on the cell surface, followed by binding to the Cy3-labeled human IgG, which shows red fluorescence; D. Images A, B, and C were merged. E–H. The results of the negative samples under fluorescence microscope; E. DAPI staining of nuclei; F. eGFP tagged at the AQP4 C-terminus showing green fluorescence for the negative sample; G. The negative samples with absent AQP4 antibodies fail to bind fluorescent secondary antibodies to the cell surface; H. Image E, F, and G were merged.

3.4. The proportion of positive samples among different age groups and genders

The total positive rate was 16.15% (362/2241), with 5.8% (57/2241) for males and 24.23% (305/2241) for females. The Chi-square test result was $\chi^2 = 138.22$, with a P -value < 0.05 , indicating a significant difference between the groups. In other words, the incidence rate among females was notably higher than that among males. The mean age of onset was 50.44 ± 14.73 , with 51.35 ± 16.82 for males and 50.28 ± 14.33 for females. The results are presented in **Table 2**.

Table 2. Positive rate statistics of the samples

Total sample size	Total positive rate (%)	Male positive rate (%)	Female positive rate (%)	χ^2	P	Mean age of onset
2241	362 (16.15%)	57 (5.8%)	305 (24.23%)	138.221	$P < 0.05$ ($6.5195E^{-32}$)	50.44 ± 14.73

3.5. Comparison with commercially available ELISA kits

Discrepancies were observed between the results obtained using the commercial kits (RSR Limited) and the CBA method for clinical samples (**Table 3**). Among the 15 groups of samples tested, four yielded differing results, and one serum sample was not detected by the commercial kits. Moreover, the results for the 3 cases of cerebrospinal fluid samples were incongruous with those obtained using the CBA method in the laboratory. To assess the concordance between the novel methodology employed in this study and the commercially available kits, the kappa test was utilized to meticulously analyze the agreement rate between the two approaches. **Table 4** provides the data about the concordance rate. The statistical analysis revealed a kappa value of 0.722, indicating a substantial level of agreement, with a P -value of 0.000, which is statistically significant.

Table 3. Test results of CBA and ELISA

Serum	Commercially ELISA kits cut off > 3.0	CBA method in the laboratory	CSF	Commercially ELISA kits cut off > 3.0	CBA method in the laboratory	Conclusion
NMO23476-1	0.30	1:10	NMO23476-2	3.13	1:1	Serum results showed inconsistencies
NMO23575-1	33.89	1:32	NMO23575-2	26.85	1:3.2	paired samples positive
NMO23582-1	20.50	1:32	NMO23582-2	2.64	1:1	CSF results showed inconsistencies
NMO23592-1	36.88	1:100	NMO23592-2	3.73	1:10	Paired samples positive
NMO23684-1	0.33	negative	NMO23684-2	2.67	negative	Paired samples negative
NMO23685-1	0.38	negative	NMO23685-2	1.22	negative	Paired samples negative
NMO23686-1	0.43	negative	NMO23686-2	2.37	negative	Paired samples negative
NMO23687-1	0.41	negative	NMO23687-2	1.43	negative	Paired samples negative
NMO23688-1	0.35	negative	NMO23688-2	2.27	negative	Negative
NMO22619-1	9.63	1:32	NMO22619-2	11.04	1:10	Paired samples positive
NMO22363-1	0.12	negative	NMO22363-2	3.25	negative	CSF results showed inconsistencies
NMO22366-1	0.15	negative	NMO22366-2	3.38	negative	CSF results showed inconsistencies
NMO22377-1	7.65	1:10	NMO22377-2	3.65	1:1	Paired samples positive
AQP22074-1	0.45	negative	AQP22074-2	2.94	negative	Paired samples negative
NMO23642-1	0.33	negative	NMO23642-2	2.59	negative	Paired samples negative

Table 4. Concordance statistics

Method 1 (CBA)	Method 2 (ELISA)		Total
	Positive	Negative	
Positive	10	2	12
Negative	2	16	18
Total	12	18	30

4. Discussion

Neuromyelitis optica (NMO) is an autoimmune central nervous system disease characterized by acute or subacute demyelination, that can affect the optic nerve and spinal cord, resulting in single or recurrent optic neuritis and transverse myelitis. Neuromyelitis optica and multiple sclerosis have several clinical manifestations. However, the principles of treatment differ significantly between the two conditions. As such, it is crucial to make an early and accurate diagnosis of neuromyelitis optica to ensure effective treatment for patients. In recent years, immunopathological research has demonstrated a significant association between antibodies against aquaporin-4 (AQP4) and the pathogenesis and development of neuromyelitis optica spectrum disorder (NMOSD). Detection of anti-AQP4 antibodies in patient serum and cerebrospinal fluid (CSF) may contribute to early diagnosis of NMOSD^[3]. The 2021 version of the “Diagnosis and Treatment Guidelines for Neuromyelitis

Optica Spectrum Disorder” proposes that AQP4-IgG antibodies are highly specific diagnostic biomarkers, with approximately 70% to 80% of NMOSD patients expressing AQP4-IgG. In 1994, Jing et al. obtained aquaporin-4 (AQP4) by homologous cloning of the aquaporin family ^[4]. The protein is located on the cell membrane, and its monomer is composed of eight transmembrane structures (M1–M8) with five ring structures. Three ring structures (A, C, and E) are located outside the cell, and two ring structures (B, D), carboxyl, and amino termini are located inside the cell. Among them, loops B and E contain highly conserved asparagine-proline-alanine (NPA) repeat tandem sequences, and the two NPA loops return to and from the bilayer of the cell membrane to form a hydrophilic channel ^[5]. AQP4 is highly expressed in astrocytes throughout the central nervous system (CNS), with a particular abundance at the interface between the brain parenchyma, cerebral ventricle, and cerebrospinal fluid in the subarachnoid space ^[3]. The primary function of AQP4 facilitate water transport between the blood and brain as well as between the brain and cerebrospinal fluid (CSF) compartments. As a bidirectional channel protein, AQP4 can promote edema formation in cases of cytotoxic edema caused by ischemia or poisoning when the blood-brain barrier remains intact. Conversely, it can help alleviate excessive hydrocephalus in cases of vasogenic and interstitial edema when the blood-brain barrier is compromised ^[6]. Further studies have confirmed that the AQP4-IgG antibody is mainly of the IgG1 isotype ^[6]. The mechanism of action of AQP4-IgG antibody in patients with neuromyelitis optica involves antibody binds to the extracellular segment of the AQP4 protein on the surface of astrocyte foot processes in a nonlinear three-dimensional conformation, activating complement, and subsequently forming a membrane attack complex to damage astrocytes. Investigations have revealed that the expression of AQP4 in the optic nerve, spinal cord, and other brain tissues is significantly elevated compared to that in peripheral tissues and can aggregate to form larger-volume composite structures that are more easily recognizable by antibodies ^[7].

There are several methods for AQP4-IgG antibody detection: tissue-based indirect immunofluorescence assay (TBA), cell-based indirect immunofluorescence assay (CBA), and enzyme-linked immunosorbent assay (ELISA). Among them, TBA and CBA methods ensure the native conformation of the protein, but the operation is relatively complicated. In terms of positive detection rate, compared with the three methods, the CBA method had the highest positive detection rate and sensitivity. Although the ELISA method is more sensitive, the specificity is reduced. The guidelines do not recommend it as a detection method to establish the diagnosis, but it has a certain value in the longitudinal monitoring of antibody titers for the evaluation of disease progression and treatment ^[3]. Chinese scholar Li Min also found that when detecting AQP4-IgG antibodies in serum and cerebrospinal fluid samples of patients with neuromyelitis optica, the positive detection rate of CBA was 73.1% and 85.1%, respectively, followed by IIF, and the positive detection rate of ELISA was lower ^[8]. The CBA assay is the preferred method for AQP4-IgG antibody detection.

This study performed an indirect immunofluorescence assay based on cytology. The method involves expressing the AQP4 protein in eukaryotic cells through cell transfection, which not only preserves the native conformation of the protein but also enhances its expression level, thereby ensuring detection sensitivity. This approach demonstrated excellent performance in detecting clinical samples in our laboratory.

Detection of AQP4-IgG antibodies has emerged as a crucial reference indicator for the diagnosis, treatment, and prognosis of neuromyelitis optica spectrum disorder (NMOSD). Enhancing the sensitivity of AQP4-IgG antibody detection has attracted significant attention from researchers. This is particularly important during the early stages of the disease, as it holds great clinical application value in assisting physicians in making precise diagnoses and providing the best possible treatment opportunities for patients.

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Disclosure statement

The authors declare no conflict of interest.

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High Ambient Temperatures Increase Outpatient Visits for Sleep Disorders in Hefei City, China

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Abstract: *Objective:* To analyze the impact of ambient temperature on the quantity of outpatient clinic visits for sleep disorders. *Methods:* Using data from sleep disorder outpatient visits in a large tertiary hospital in Hefei City, a distributional lag nonlinear model combined with a generalized Poisson regression model was used to analyze the relationship between ambient temperature and the number of outpatient visits for sleep disorders. *Results:* Ambient temperatures above 17.2°C were found to be connected with a higher prevalence of sleep disorders visits, and that this relationship was most significant on day 8, which lasted for 7 days. For the single-day lagged impact, the maximum relative risk (RR) for moderate heat (75th percentile) was 1.077 (95% CI: 1.015–1.143). The cumulative lag effect was substantially greater than the single-day lag effect, with a maximum relative risk (RR) of 2.609 (95% CI: 1.306–5.212). The longest lag time was 14 days. The RR was similarly greater in women and those over 40. Outpatient visits for men with sleep disorders were not affected by ambient temperature in a statistically significant way. *Conclusion:* High ambient temperature raises the risk that patients will visit an outpatient facility and serves as a risk factor for sleep disorders. Patients who were 40 years of age or older and women were at vulnerability.

Keywords: Ambient temperature; Sleep disorders; Time-series analysis

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1. Introduction

Sleep disorders are characterized by insufficient sleep, excessive sleep, or irregular movements during sleep. Sleep disorders affect 0.047% to 50.5% of the population, with insomnia being the most prevalent type, followed by sleep breathing disorders, restless legs syndrome, nightmares, sleepwalking, and narcolepsy^[1]. Sleep disorders can alter immune mechanisms like neuroendocrine thermoregulation, leading to a pathological process^[2–3]. It not only raises the risk of hypertension, diabetes, heart disease, dementia, and stroke, but it also has significant psychological and economic effects^[4].

Sleep quality is closely connected to ambient temperature and humidity ^[5]. High ambient temperatures have a negative impact on sleep quality ^[6]. Ambient temperature was the most important element influencing sleep quality, with relative humidity and lighting having negligible effects ^[7]. The global temperature rise will exceed 1.5°C between 2030 and 2052, according to the Intergovernmental Panel on Climate Change, and weather extremes will become more violent and frequent ^[8]. It is imperative to assess the detrimental effects of ambient temperature on health and adopt effective healthcare initiatives and laws to prevent and lessen these effects in light of the constantly changing environment. As a result, this study will statistically examine the association between ambient temperature and outpatient visits of sleep disorders in Hefei City from 2018 to 2020 to analyze the influence of short-term temperature variations on population health.

2. Materials and methods

2.1. Study area

Hefei, Anhui Province in China, is set in eastern China and the western end of the Yangtze River Delta. It has four distinct seasons, a pleasant environment, and moderate rainfall.

2.2. Date collection

Between January 1, 2018, and December 31, 2020, the study gathered information on daily outpatient attendances for sleep disorders at three campuses of a sizable tertiary care hospital. The age, gender, and dwelling address were among the basic details. To clarify the diagnosis, sleep disorders were described using the International Classification of Diseases (ICD-10), and diagnostic information was documented by an outpatient electronic healthcare record system. Individuals who (1) did not know when their symptoms started, (2) did not have a fixed address or did not live in Hefei, (3) had a questionable diagnosis, and (4) were not sure of their gender or age, were not included in the study.

The Hefei Meteorological Bureau provided meteorological data from 1 January 2018 to 31 December 2020. Meteorological monitoring procedures have been implemented to meet Chinese surface meteorological observation criteria. The study collected the daily mean temperature (T_{mean}), relative humidity (RH), wind speed (WS), sunshine hours (SH), and precipitation from the Meteorological Bureau's statistics. By deducting the daily highest temperature from the daily minimum temperature, the diurnal temperature range (DTR) was obtained. The Hefei Environmental Monitoring Centre provided air pollution statistics for the same period.

2.3. Statistical analysis

This study utilized a generalized Poisson regression model together with a distributional lag nonlinear model (DLNM) to quantitatively examine the impacts of ambient temperature on sleep disorder outpatient attendances owing to the “over-dispersed” distribution of sleep disorder outpatients. After compiling the consultation and meteorological data with SPSS 25.0, statistical analysis was completed using R software (version 4.2.2). Using a two-sided test, $\alpha = 0.05$. Spearman correlation studies were conducted for meteorological elements and air pollutants to prevent multicollinearity between variables. Correlation coefficients greater than 0.6 were excluded from the model. The model included rainfall, PM_{2.5}, DTR, and SO₂. To evaluate degrees of freedom and model fit, the specific model was constructed using residual analysis and the Akaike Information Criterion (AIC):

$$Y_t \sim \text{quasi-Poisson}(\mu_t)$$

$$\text{Log}(\mu_t) = \alpha + \beta T_{\text{meant},t,3} + \text{ns}(\text{DTR}_{t,1,3}) + \text{ns}(\text{SO}_{2,t,1,3}) + \text{ns}(\text{PM}_{2.5,t,1,3}) + \text{ns}(\text{Rainfall}_{t,1,3}) + \text{ns}(\text{Timet},7) + \gamma \text{Dow}_t + \delta \text{Holiday}_t$$

t and l stand for the visitation date and lag days, respectively; On day t, Y_t is the anticipated total number of outpatient appointments for sleep disorders; The mean temperature's DLNM cross-base function is $T_{mean,t,l}$; $\text{Log}()$ represents the link function, and α indicates intercepting. The T_{mean} index coefficient is represented by β , while the Dow and Holiday matrices factors, each representing weekday and holiday effects, are represented by γ and δ , respectively. The natural cubic spline function is indicated by ns; and $DTR_{t,l}$, with three degrees of freedom, stands for the daily comparative difference on day t; ($DTR_{t,l,3}$), ($SO2_{t,l,3}$), ($PM2.5_{t,l,3}$), (Rainfall t,l,3) with a degree of freedom of 3. $\text{Time}_{t,7}$ indicates the time tendency with seven degrees of freedom. The study examined the lagged effect from 1 to 14 days with a mod_t lag date of 14 days. The relative risk (RR) and 95% confidence interval (CI) were used to analyze the relationship between T_{mean} and sleep disorders outpatient visits.

Using the median corresponding temperature of 17.2°C as a reference, the relative risk (RR) of mild hyperthermia (75th percentile) was calculated for this investigation. Potential impacts of heat correction resulting from certain patient characteristics were analyzed by age group (0–40 years, ≥ 40 years), as well as sex (male and female). Additionally, the study performed many analyses of sensitivity and adjusted the rainfall (df = 3-5), and SO2 (df = 3-5).

3. Results

3.1. Descriptive statistics

Table 1 displays daily data from sleep disorder clinic visits along with air pollution and weather information. For sleep disorders, there were 2796 outpatient consultations in total during the study interval. There were 1736 cases of females (62%) and 71.6% of the population is ≥ 40 years of age.

Table 1. Statistical characteristics of outpatient visits for weather variables and sleep disorders in Hefei, 2018–2020

Group	Sum	(Mean \pm SD)	Min	P5	P25	P50	P75	P95	Max
All visits	2796	2.55 \pm 2.46	0	0	1	2	4	8	14
Male	1060	0.97 \pm 1.14	0	0	0	1	2	3	6
Female	1736	1.58 \pm 1.79	0	0	0	1	2	5	10
< 40years	793	0.72 \pm 1.01	0	0	0	1	3	5	11
≥ 40 years	2003	1.83 \pm 1.96	0	0	0	0	1	3	7
T_{mean} (°C)	-	16.55 \pm 9.42	-5.3	1.4	8.2	17.2	24.5	30	33.3
DTR (°C)	-	9.24 \pm 4.57	0.6	2.2	5.7	9.1	12.5	17	21.5
RH (%)	-	78.56 \pm 11.80	35	58	71	80	87	96.3	99
WS (m/s)	-	2.12 \pm 0.88	0.4	1.0	1.5	2.0	2.6	3.8	6.2
SH (h)	-	5.19 \pm 4.30	0	0	0	5.7	9.1	11.5	12.9
Rainfall (mm)	-	3.29 \pm 11.55	0	0	0	0	0.7	18.1	197.4
PM2.5 ($\mu\text{g}/\text{m}^3$)	-	42.92 \pm 27.97	5	13	24	35	53	103	184
NO ₂ ($\mu\text{g}/\text{m}^3$)	-	40.69 \pm 17.82	10	18	27	37	52	74	102
SO ₂ ($\mu\text{g}/\text{m}^3$)	-	6.67 \pm 3.02	2	3	5	6	8	13	27
O ₃ ($\mu\text{g}/\text{m}^3$)	-	112.9 \pm 49.64	6	39.6	74	110	146	197	255

3.2. Analysis of the correlation of significant environmental elements

Figure 1 depicts the Spearman's correlation between the major environmental elements that are associated with

sleep disorders. The mean ambient temperature was inversely associated with WS, PM2.5, NO₂, and SO₂, but positively related to DTR, sunshine hours, and rainfall.

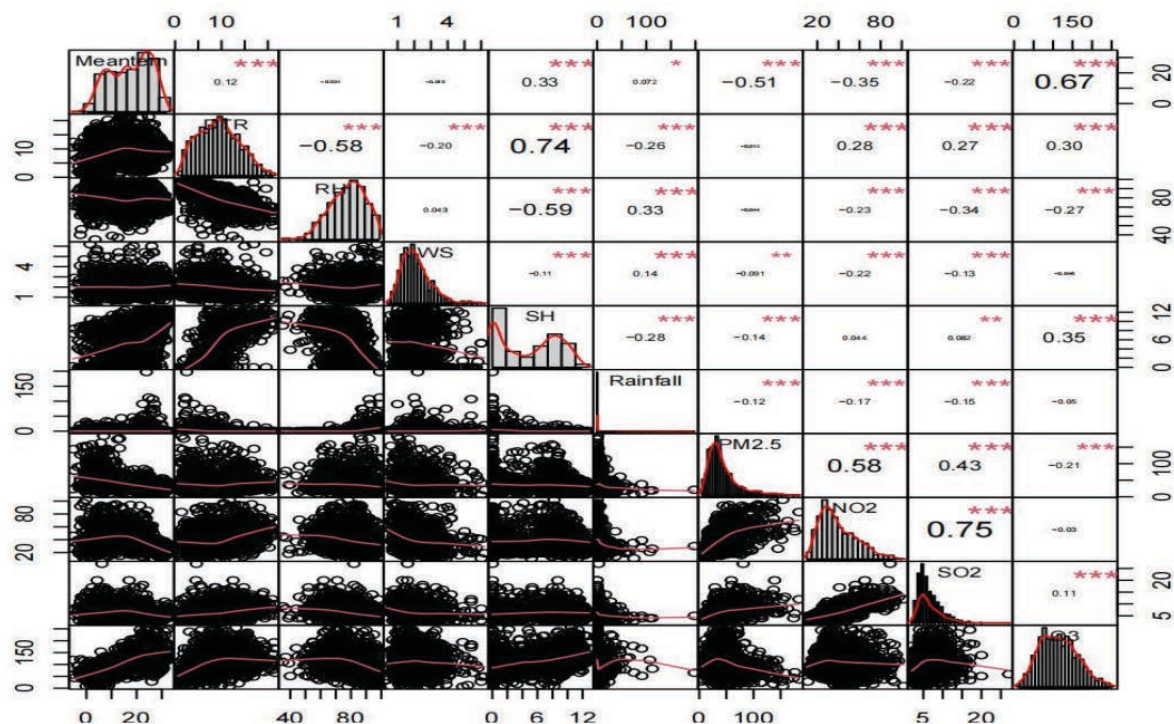


Figure 1. Shows the Spearman's correlation coefficients between main pollutants and meteorological conditions; * $P < 0.05$, with a scatterplot at the bottom and Spearman's correlation coefficients at the top

3.3. Mean temperature's effect on outpatient visits for sleep disorders

The entire exposure-response connection between mean temperature and sleep disorder clinic visits is depicted in **Figure 2**. The average temperature rose above 17.2°C, and a significant increase in patients visiting sleep disorder clinics occurred. It seems that lowering the temperature guards against insomnia. The delayed effect and the longest lag time of 14 days are shown in **Figure 2**.

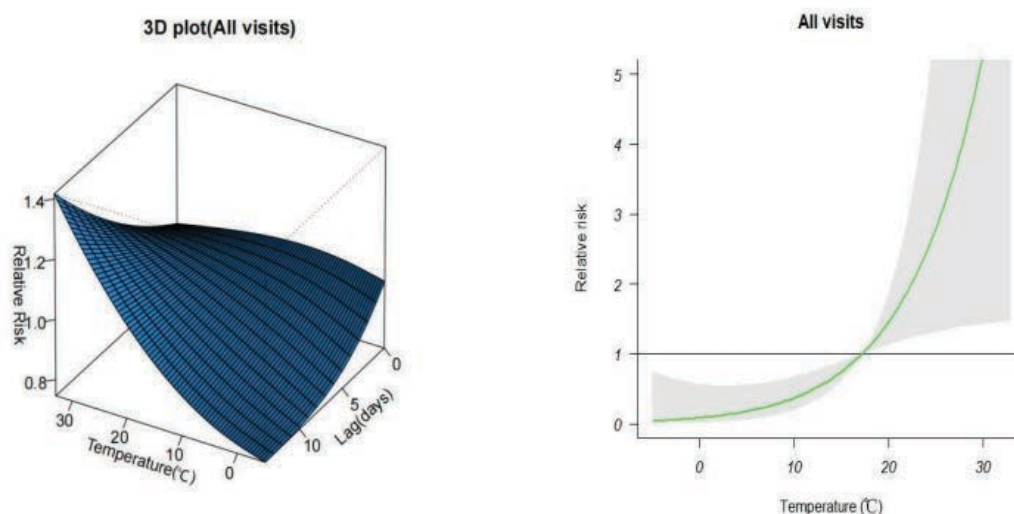


Figure 2. Exposure-response relationship between mean temperature and sleep disorder attendees in the Hefei

3.4. Lagged impacts of certain mean temperatures

Table 2 and **Table 3** (reference 17.2°C) present the single-day and cumulative lagged impacts of mean temperatures on outpatient visits related to sleep disorders. The impact of high temperatures on sleep disorder visits is significant. The impact of high temperatures on visits related to sleep disorders is noteworthy. The cumulative lag impact was substantially greater than the single-day lag impact, starting on day 12 with an RR of 1.943 (95% CI: 1.080–3.495) and lasting for 6 days to reach its maximum. The single-day lag influence started on the 8th day with an RR of 1.077 (95% CI: 1.015–1.143). The lagged impact for females began on the 8th day with an RR of 1.077 (95% CI: 1.002–1.158) and persisted for 6 days, whereas the cumulative lagged effect began on day 12 and lasted for 3 days, reaching a maximum with an RR of 2.937 (95% CI: 1.265–6.821). In men, the delayed effect began on day 11 and lasted for two days, with an RR of 1.105 (95% CI: 1.006–1.213). Age analysis revealed that the lag impact began on day 10 and continued only 3 days in patients <40 years with sleep difficulties, with an RR of 1.132 (95% CI: 1.001–1.280) and no significant cumulative lag impact. In contrast, patients older than 40 years had a lag effect that was comparable to that of the population in general (RR = 1.081, 95% CI: 1.009–1.158). Hypothermia dramatically reduced the probability of outpatient visits for sleep disturbances in women and people of ≥40 years. In men and the <40 years group, however, it was not statistically significant. The results are illustrated in **Figure 3**.

Table 2. The single-day lagged effect of high temperature on outpatient visits for sleep disorders, reference 17.2°C

Single-day (day(s))	Relative risk (95% confidence interval)				
	Total	Male	Female	<40 years	≥40 years
Lag0	0.979 (0.859– 1. 117)	0.929 (0.771– 1. 119)	1.011 (0.863– 1. 184)	0.961 (0.787– 1. 173)	0.983 (0.845– 1. 144)
Lag1	0.990 (0.901– 1.089)	0.949 (0.829– 1.086)	1.014 (0.905– 1. 137)	0.974 (0.843– 1. 125)	0.995 (0.891– 1. 110)
Lag2	1.001 (0.935– 1.073)	0.969 (0.879– 1.068)	1.018 (0.937– 1. 107)	0.988 (0.890– 1. 109)	1.007 (0.929– 1.091)
Lag3	1.013 (0.956– 1.074)	0.987 (0.910– 1.072)	1.024 (0.954– 1.099)	1.001 (0.918– 1.093)	1.019 (0.952– 1.091)
Lag4	1.025 (0.966– 1.087)	1.005 (0.926– 1.091)	1.031 (0.960– 1. 108)	1.015 (0.930– 1. 108)	1.031 (0.962– 1. 105)
Lag5	1.037 (0.975– 1.004)	1.022 (0.937– 1. 114)	1.041 (0.964– 1. 123)	1.029 (0.938– 1. 129)	1.043 (0.970– 1. 122)
Lag6	1.050 (0.985– 1. 120)	1.037 (0.949– 1. 134)	1.051 (0.972– 1. 136)	1.044 (0.949– 1. 148)	1.056 (0.980– 1. 137)
Lag7	1.064 (0.999– 1. 132)	1.052 (0.964– 1. 149)	1.063 (0.985– 1. 148)	1.058 (0.963– 1. 162)	1.068 (0.993– 1. 149)
Lag8	1.077 (1.015– 1. 143)*	1.066 (0.980– 1. 160)	1.077 (1.002– 1. 158)*	1.072 (0.980– 1. 174)	1.081 (1.009– 1. 158)*
Lag9	1.091 (1.031– 1. 155)*	1.079 (0.995– 1. 171)	1.091 (1.019– 1. 169)*	1.087 (0.997– 1. 186)	1.093 (1.024– 1. 168)*
Lag10	1.037 (0.954– 1. 126)*	1.092 (1.005– 1. 187)*	1. 107 (1.032– 1. 187)*	1. 102 (1.008– 1.205)*	1. 106 (1.035– 1. 183)*
Lag11	1. 120 (1.049– 1. 197)*	1. 105 (1.006– 1.213)*	1. 124 (1.037– 1.217)*	1. 117 (1.009– 1.236)*	1. 119 (1.037– 1.207)*
Lag12	1. 135 (1.048– 1.230)*	1. 117 (0.997– 1.250)	1. 141 (1.035– 1.258)*	1. 132 (1.001– 1.280)*	1. 132 (1.031– 1.243)*
Lag13	1. 151 (1.041– 1.272)*	1. 128 (0.981– 1.297)	1. 159 (1.026– 1.309)*	1. 147 (0.986– 1.336)	1. 145 (1.020– 1.286)*
Lag14	1. 166 (1.032– 1.318)*	1. 140 (0.962– 1.352)	1. 177 (1.014– 1.368)*	1. 163 (0.966– 1.400)	1. 159 (1.005– 1.336)*

* $P < 0.05$

Table 3. The cumulative lagged effect of high temperature on outpatient visits for sleep disorders, reference 17.2°C

Multi-day (day(s))	Relative risk (95% confidence interval)				
	Total	Male	Female	<40 years	≥40 years
Lag0-0	0.979 (0.859–1.117)	0.929 (0.771–1.119)	1.011 (0.863–1.184)	0.961 (0.787–1.173)	0.983 (0.845–1.144)
Lag0-1	0.970 (0.775–1.215)	0.882 (0.641–1.214)	1.026 (0.782–1.345)	0.936 (0.665–1.318)	0.978 (0.754–1.269)
Lag0-2	0.972 (0.730–1.296)	0.855 (0.568–1.286)	1.045 (0.739–1.478)	0.925 (0.598–1.432)	0.985 (0.707–1.374)
Lag0-3	0.985 (0.709–1.369)	0.845 (0.529–1.347)	1.071 (0.720–1.591)	0.927 (0.563–1.527)	1.005 (0.687–1.470)
Lag0-4	1.010 (0.706–1.446)	0.849 (0.510–1.413)	1.105 (0.717–1.703)	0.942 (0.547–1.621)	1.036 (0.684–1.571)
Lag0-5	1.049 (0.714–1.540)	0.868 (0.504–1.496)	1.150 (0.722–1.831)	0.970 (0.543–1.733)	1.082 (0.692–1.691)
Lag0-6	1.102 (0.731–1.662)	0.901 (0.504–1.609)	1.210 (0.735–1.991)	1.013 (0.546–1.878)	1.143 (0.708–1.843)
Lag0-7	1.173 (0.756–1.818)	0.948 (0.511–1.759)	1.287 (0.755–2.193)	1.072 (0.555–2.069)	1.221 (0.732–2.036)
Lag0-8	1.264 (0.791–2.014)	1.012 (0.524–1.951)	1.386 (0.784–2.450)	1.150 (0.571–2.314)	1.320 (0.765–2.278)
Lag0-9	1.380 (0.839–2.267)	1.093 (0.544–2.193)	1.514 (0.826–2.773)	1.251 (0.596–2.626)	1.444 (0.809–2.578)
Lag0-10	1.526 (0.903–2.580)	1.194 (0.572–2.493)	1.676 (0.884–3.179)	1.379 (0.630–3.020)	1.599 (0.867–2.948)
Lag0-11	1.710 (0.983–2.977)	1.320 (0.606–2.871)	1.884 (0.959–3.702)	1.541 (0.673–3.527)	1.790 (0.939–3.413)
Lag0-12	1.943 (1.080–3.495)*	1.474 (0.646–3.363)	2.151 (1.051–4.399)*	1.745 (0.724–4.208)	2.027 (1.023–4.015)*
Lag0-13	2.236 (1.190–4.202)*	1.664 (0.686–4.039)	2.494 (1.157–5.376)*	2.004 (0.776–5.172)	2.323 (1.116–4.835)*
Lag0-14	2.609 (1.306–5.212)*	1.899 (0.718–5.023)	2.937 (1.265–6.821)*	2.332 (0.821–6.621)	2.693 (1.206–6.013)*

* $P < 0.05$

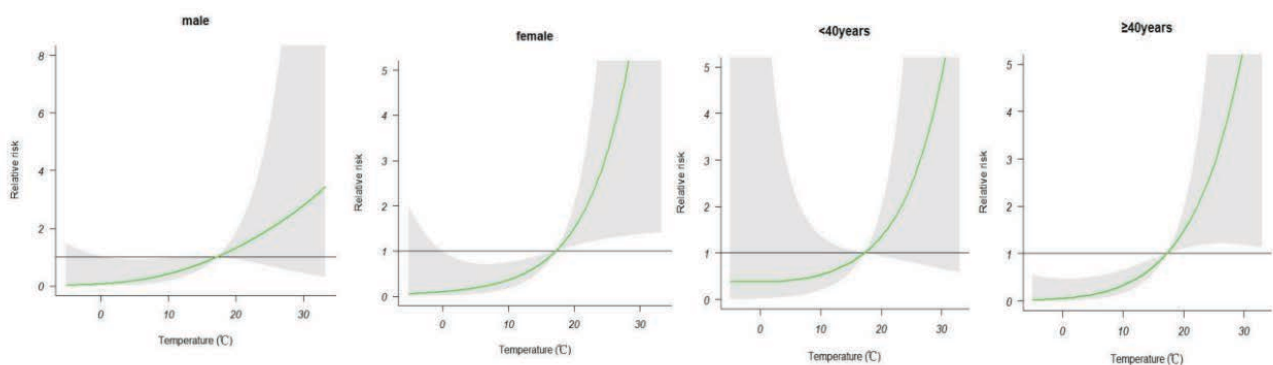


Figure 3. Exposure-response relationships between sleep disorders visit and mean temperature in a subgroup of patients, in Hefei City

3.5. Sensitivity analyses

The sensitivity analyses demonstrate that for the SO_2 ($df = 3-5$) and rainfall ($df = 3-5$), the exposure-response

curves are consistent throughout degrees of freedom in the model. It indicates the stability of the model, as illustrated in **Figure 4** and **Figure 5**.

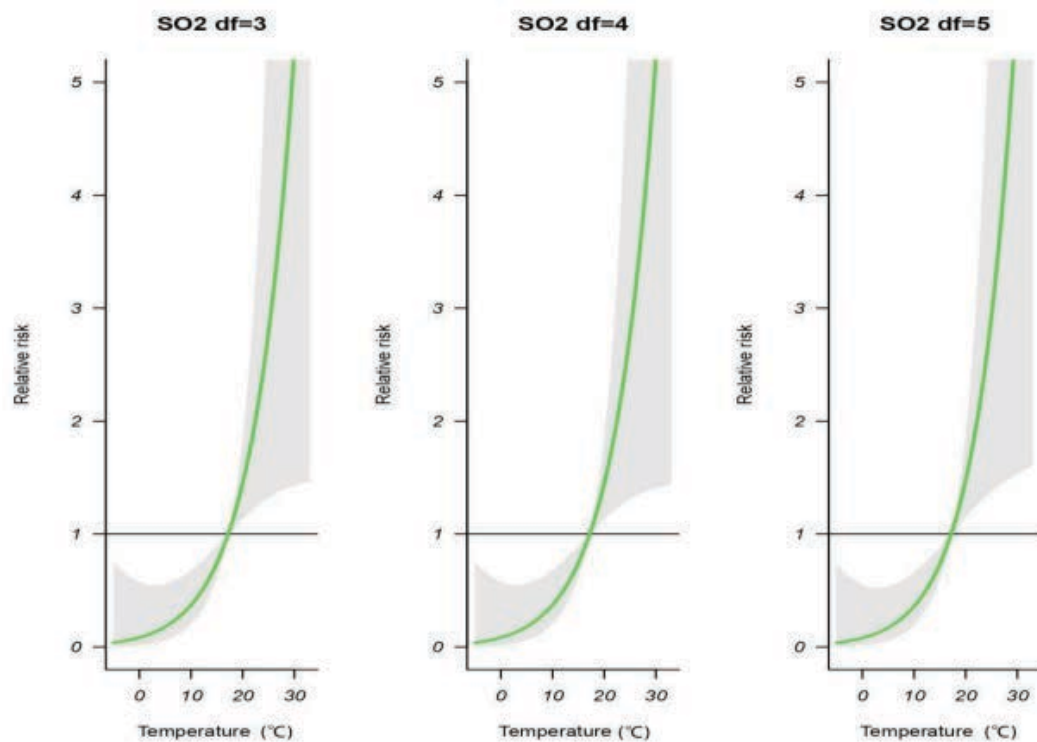


Figure 4. Sensitivity analysis when altering the degrees of freedom (df = 3–5) for SO₂ in the model

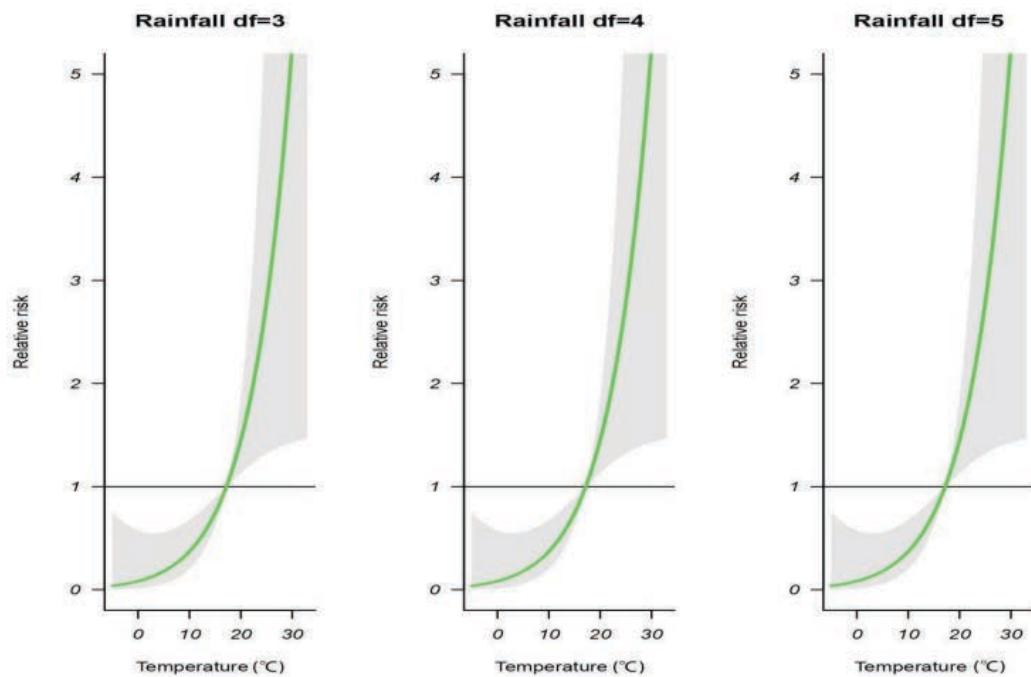


Figure 5. Sensitivity analysis when altering the degrees of freedom (df = 3–5) for rainfall in the model

4. Discussion

The research used a generalized Poisson regression model in conjunction with a distributional lag nonlinear model to examine the relationship between ambient temperature and sleep disorders. The study discovered a substantial positive relationship between the occurrence of outpatient visits for sleep disorders and ambient temperature above 17.2°C. This effect was significant starting at 8 days lag and lasting for 7 days. Low temperatures are protective against sleep disorders. The results show that elevated high temperature is associated with an increased risk of sleep disorders. Furthermore, the study discovered that women and people over the age of 40 were more vulnerable to the impacts of high temperatures.

A considerable amount of research supports the assumption that high temperatures are a favorable factor in the development of sleep disorders^[9]. The prevalence of sleep deficit rises in high temperatures^[10–11]. The precise mechanism by which temperature contributes to sleep disorders is unknown at this time, although the link seems biologically reasonable. The effects of direct exposure to hot surroundings during the day, such as heat stress, cardiovascular stress, or dehydration, may persist throughout the night^[12]. As a result, being exposed to greater ambient temperatures may impair the body's capacity to regulate its own temperature and make falling asleep harder, leading to sleep disorders^[13]. A 68-country research of ambient temperature on sleep measurements discovered that greater nighttime temperatures lowered sleep duration. This is congruent with the findings of the study, which discovered that high ambient temperatures increased outpatient visits for sleep disorders^[14–16].

Based on age and gender, the research found differences in vulnerability to ambient temperature impacts on sleep disorders. High temperatures had a stronger impact on outpatient visits for sleep disturbances in people over 40 and women. A study conducted in the United States discovered a strong connection between overnight temperatures and sleep disorders, this correlation was most pronounced in the summer and among older adults and those with poor incomes^[17]. Potential pathways include body skin temperature related to older persons and sleep outcomes, according to research conducted in Japan and China, with greater skin temperatures in older populations associated with poorer sleep quality^[18]. Another study discovered that the higher the difference between daytime and nighttime temperatures, the greater the sleep loss, and the longer the cumulative lag impact of outside ambient temperature on sleep reduction, demonstrating that indoor environments might hold ambient heat and improve heat-related sleep deprivation^[19]. As for gender differences, they might be connected to physiological elements (such as higher subcutaneous fat content, larger area of body surface-to-fat content ratios, and hormone adjustments) that make females more susceptible to high temperatures^[20].

5. Conclusions

High ambient temperature may be a risk factor for sleep disorders, people ≥ 40 years, and women with sleep disorders are vulnerable to high ambient temperatures. When hot weather is forecast, vulnerable people should be reminded to take precautions as soon as possible and to remain watchful for 8 days after exposure. Furthermore, sleep problem clinics should boost physician scheduling during peak times to better allocate medical resources.

Disclosure statement

The authors declare no conflict of interest.

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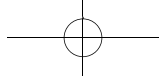
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