OPEN

# Low expression of thiamine pyrophosphokinase-1 contributes to brain susceptibility to thiamine deficiency

Yingfeng Xia[a,*], Ting Qian[a,b,*], Guoqiang Fei[a], Xiaoqin Cheng[a], Lei Zhao[c,*], Shaoming Sang[a,*] and Chunjiu Zhong[a]

**Thiamine deficiency is a well-known risk factor for the development of severe encephalopathy, such as Wernicke encephalopathy and Korsakoff syndrome, but the underlying mechanism is still mysterious. This study aims to investigate the expression levels of thiamine metabolism genes in different tissues and their impact on brain susceptibility to thiamine deficiency. The mRNA and protein levels of four genes known to be associated with thiamine metabolism: thiamine pyrophosphokinase-1 (*Tpk*), *Solute carrier family 19 member 2* (*Slc19a2*), *Slc19a3*, and *Slc25a19*, in the brain, kidney, and liver of mice were examined. Thiamine diphosphate (TDP) levels were measured in these tissues. Mice were subjected to dietary thiamine deprivation plus pyrithiamine (PTD), a specific TPK inhibitor, or pyrithiamine alone to observe the reduction in TDP and associated pathological changes. TPK mRNA and protein expression levels were lowest in the brain compared to the kidney and liver. Correspondingly, TDP levels were also lowest in the brain. Mice treated with PTD or pyrithiamine alone showed an initial reduction in brain TDP levels, followed by reductions in the liver and kidney. PTD treatment caused significant neuron loss, neuroinflammation, and blood–brain barrier disruption, whereas dietary thiamine deprivation alone did not. TPK expression level is the best indicator of thiamine metabolism status. Low TPK expression in the brain appears likely to contribute to brain susceptibility to thiamine deficiency, underscoring a critical role of TPK in maintaining cerebral thiamine metabolism and preventing thiamine deficiency-related brain lesions.** *NeuroReport* **35: 1000–1009 Copyright © 2024 The Author(s). Published by Wolters Kluwer Health, Inc.**

**NeuroReport** 2024, **35**:1000–1009

**Keywords: brain susceptibility, encephalopathy, thiamine deficiency, thiamine pyrophosphokinase**

[a]Department of Neurology, Zhongshan Hospital; State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science; Institutes of Brain Science; National Clinical Research Center for Aging and Medicine, Huashan Hospital; Fudan University, [b]Department of Neurology, Shanghai Tenth People's Hospital, Tongji University School of Medicine and [c]Department of Neurology, Ninth People's Hospital, Shanghai Jiao Tong University, Shanghai, China

Correspondence to Chunjiu Zhong, PhD, Department of Neurology, Zhongshan Hospital; State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science; Institutes of Brain Science; National Clinical Research Center for Aging and Medicine, Huashan Hospital; Fudan University, Shanghai 200032, China
Tel: +86 13901809740; e-mail: zhongcj@163.com

*Lei Zhao, Shaoming Sang, Yingfeng Xia and Ting Qian contributed equally to the writing of this article.

**Received** 23 July 2024 **Accepted** 8 August 2024.

## Introduction

Thiamine diphosphate (TDP), the active form of thiamine, is the cofactor of transketolase, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched chain α-ketoacid dehydrogenase. Transketolase is involved in the non-oxidative branch of the pentose phosphate pathway. Pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched chain α-ketoacid dehydrogenase are mitochondrial enzymes critical for oxidative decarboxylation of pyruvate, α-ketoglutarate, or branched-chain amino acids. They play an essential role in glucose metabolism [1,2]. Abnormal thiamine metabolism characterized by TDP reduction induced by genetic mutations, improper medical treatment, or unhealthy lifestyles significantly predisposes to brain damage, causing Wernicke encephalopathy (WKS), Korsakoff syndrome, and biotin-responsive basal ganglia disease. These effects are attributed to disrupted TDP-dependent metabolic processes, which are involved in energy metabolism and neurotransmitter synthesis [2]. Our and other studies have also found that thiamine deficiency is involved in several neurodegenerative diseases, including Alzheimer's disease, through activating a cascade of energy deficiency, oxidative stress, endoplasmic reticulum stress, neuroinflammation, and ultimately neuron death [3–7]. Although the maintenance of normal function of all other organs is also strictly dependent on TDP, the brain is particularly vulnerable to thiamine deficiency [8,9]. Clarifying the underlying mechanism by which the brain is vulnerable to thiamine deficiency will promote our understanding of the homeostasis of thiamine metabolism in the tissues and help discover new therapeutic options for brain diseases associated with disrupted homeostasis of thiamine metabolism.

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.neuroreport.com.

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Thiamine cannot be synthesized endogenously in mammals and has to be absorbed from food. Currently, four genes are known to be associated with thiamine metabolism: *Solute carrier family 19 member 2* (*SLC19A2*), *SLC19A3*, *thiamine pyrophosphokinase-1* (*TPK*), and *SLC25A19*. The first two encode thiamine transporter-1 (THTR-1) and THTR-2, which are located on the cell membrane and mediate thiamine entry into cells and across the blood–brain barrier (BBB). Mutations of *SLC19A2* and *SLC19A3* result in thiamine-responsive megaloblastic anemia and biotin-responsive basal ganglia disease, respectively [10–13]. *TPK* is located in the cytoplasm and converts thiamine into functional TDP. Defects of *TPK* cause thiamine metabolism dysfunction syndrome-5, an encephalopathy that can be fatal in infants and young children if not treated [14,15]. *SLC25A19* encodes the mitochondrial TDP transporter that is responsible for transporting TDP into mitochondrion. The defect of *SLC25A19* leads to Amish lethal microcephaly [16]. Chronic alcoholic individuals are at a high risk of thiamine deficiency, which disrupts thiamine absorption from the gastrointestinal tract, thiamine storage in the liver, and the phosphorylation of thiamine to TDP [17]. These results indicate that thiamine transporters and metabolic enzymes play different roles in TDP homeostasis in different tissues.

For the present study, mice with dietary thiamine deprivation combined with intraperitoneal injection of pyrithiamine were selected as a well-established experimental model that excellently recapitulates the clinical features of WKS [18]. A previous study demonstrated pyrithiamine is a specific and competitive inhibitor of TPK, giving a Ki value of 19 mM. Other thiamine metabolism inhibitors such as oxythiamine and chloroethylthiamine, which are both very weak inhibitors of the enzyme, were unable to replicate human thiamine deficiency-related disorders when administered to animals [19]. Those observations suggest that TPK may play a crucial role in mediating thiamine deficiency-induced brain susceptibility. We hypothesized that differences in TPK expression may underlie thiamine deficiency-induced brain susceptibility. To address these issues, we detected the mRNA levels of four known genes associated with thiamine metabolism: *Tpk*, *Slc19a2*, *Slc19a3*, and *Slc25a19* in the different organs of wild-type mice. TPK protein level was also detected in different organs by Western blot. Thiamine and its metabolites were analyzed in different organs of control, PTD (dietary thiamine deprivation with pyrithiamine treatment), and pyrithiamine-treated mice. Brain pathological changes were compared in PTD and control mice. The results show that the levels of TPK mRNA and protein were the lowest, as is the TDP level, in the brain tissue, and we propose that this contributes to brain susceptibility to thiamine deficiency.

## Materials and methods

### Animals

All animal care and experimental procedures were performed with the approval of the Medical Experimental Animal Administrative Committee of Fudan University. Two-month-old C57BL/6 male mice were used for thiamine deficiency (TD), PTD, and pyrithiamine administration experiments. All animals were group-housed under standard laboratory conditions with humidity- and temperature-controlled environments, 12-h light/dark cycles, and free access to food and water. TD mice were produced by feeding a thiamine-depleted diet (Bio-serv Company, Flemington, New Jersey, USA). PTD mice were generated by combining a thiamine-depleted diet (Bio-Serv Company, USA) with intraperitoneal injections of 500 μg/kg/day pyrithiamine. Pyrithiamine mice were produced by administering intraperitoneal injections of 2500 μg/kg/day pyrithiamine. Control animals received a thiamine-containing diet (Bio-serv Company, USA) and distilled water ad libitum.

### RNA isolation and reverse transcription-PCR

Briefly, the total RNA of heart, liver, lung, kidney, and brain tissues of wild-type mice was extracted using TRIzol reagent (15596026CN; Invitrogen, Waltham, Massachusetts, USA) according to the manufacturer's protocol. RNA was reverse-transcribed to cDNA using ABScript II cDNA First-Strand Synthesis Kit (RK20400; Abclonal, Wuhan, China) with random primers according to the manufacturer's protocol. The qPCR assay was conducted using the TB Green Premix Ex Taq II FAST qPCR system (CN830A; Takara, Kyoto, Japan). Each sample was normalized to the *Actb* levels. The primer sequences of each gene are listed in Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/WNR/A782*.

### Western blot

Western blotting was performed as previously reported. Briefly, tissues were lysed with RIPA buffer supplemented with protease inhibitors, sonicated twice, and resuspended in 5× sample buffer (P0015L; Beyotime, Shanghai, China) supplemented with 10% beta-mercaptoethanol (M6250; Sigma-Aldrich, Darmstadt, Germany). The Pierce BCA protein assay kit (A55866; Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to determine protein concentration; 40 μg of protein was loaded on a 10% SDS-polyacrylamide gel electrophoresed and transferred to nitrocellulose (IPVH00010; Millipore, Billerica, Massachusetts, USA). The membrane was blocked overnight in 5% nonfat milk in tris-buffered saline with tween 20 at 4 °C. Primary antibodies used were: anti-TPK (ab230263, 1 : 1000; Abcam, Cambridge, UK), and anti-tubulin (2146, 1 : 10 000; Cell Signaling Technology, Danvers, Massachusetts, USA). The secondary antibodies goat anti-rabbit-HRP (BL003A, 1 : 5000;

Biosharp, Shanghai, China) were used, and images were captured using a chemiluminescence image scanner (Qingxiang, Shanghai, China). Relative grayscale value was calculated by correcting with each Coomassie staining grayscale value. These relative grayscale values are then normalized to the values of brain tissue.

#### Thiamine diphosphate, thiamine monophosphate, and thiamine

Fresh whole blood was collected from mice by cardiac puncture, anticoagulated with heparin, and deproteinized with an equal volume of 7.6% perchloric acid. Brain, kidney, and liver tissue from mice were homogenized with 100 mM of $K_2HPO_4$ (pH = 5.0) and deproteinized with an equal volume of 7.2% perchloric acid. All samples were centrifuged and supernatants were collected. The levels of TDP, thiamine monophosphate (TMP), and thiamine were measured as previously described [6]. Briefly, thiamine and its phosphate esters were derived into thiochromes using potassium ferricyanide and separated by gradient elution with a C18 reversed-phase analytical column (250 × 4.6 mm). The derivatives were measured by HPLC fluoroscopy (Agilent 1100, Santa Clara, California, USA) with an excitation wavelength of 367 nm and an emission wavelength of 435 nm. Blood TDP, TMP, and thiamine levels were quantified using standard samples of TDP, TMP, and thiamine (Sigma-Aldrich, St. Louis, Missouri, USA).

#### Immunohistochemical staining

Mice were deeply anesthetized with 0.14 g/kg sodium pentobarbital administered by intraperitoneal injection, then transcardially perfused with PBS and fixed using 4% paraformaldehyde. Serial coronal sections (35 μm) were cut with a sliding microtome (Leica, Wetzlar, Germany) and stained as freely floating sections. After washing in PBS at pH 7.4, sections were blocked in PBS containing 5% BSA and 0.5% Triton X-100 for 2 h at 37 °C, then were incubated with NeuN (1 : 500; Millipore), GFAP (1 : 500; Wako, Osaka, Japan), IBA1 (1 : 500; Wako), and mouse IgG (1 : 500; Invitrogen), overnight at 4 °C. After further PBS washing, sections were incubated with goat anti-mouse antibody conjugated to Alexa Fluor 488 and Alexa Fluor 546 (1 : 500; Invitrogen) for 2 h at 37 °C and mounted on 3-aminopropyltriethoxysilane-coated glass slides. Z-stack images were taken using Nikon A1 (Tokyo, Japan) laser scanning microscope, with a 10× objective. Results were quantified using ImageProPlus (Media Cybernetics, Silver Spring, Maryland, USA).

#### Statistical analysis

GraphPad Prism 7 (version 7.01; GraphPad software, GraphPad Software, Inc, San Diego, California, USA) was used for statistical analyses. Student's *t*-test for single comparisons or one-way ANOVA for multiple comparisons with appropriate Tukey's or Dunnett's multiple comparison tests were used to identify statistical differences. The results shown represent the mean ± SEM. '*N*' refers to the number of animals, unless otherwise indicated. All conditions that were statistically different from its control are indicated, where * represents $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** represents $P < 0.0001$.

## Results

#### Expression levels of *Tpk*, *Slc19a2*, *Slc19a3*, and *Slc25a19* in different tissues

Detection of the mRNA levels for *Tpk*, *Slc19a2*, *Slc19a3*, and *Slc25a19* in different organs of wild-type mice showed that the mRNA for *Tpk* was highest in kidney, followed by lung and liver but lowest in the brain and heart (Fig. 1a). The mRNA for *Slc19a2* was highest in the liver, followed by the lung, the kidney, the brain, and lowest in the heart (Fig. 1b). The mRNA for *Slc19a3* was highest in the kidney, followed by the brain, and lung, and lowest in the liver and the heart (Fig. 1c). The mRNA for *Slc25a19* was highest in the kidney, followed by the brain and the liver, and lowest in the lung and heart (Fig. 1d).

#### The level of brain thiamine pyrophosphokinase-1 protein is the lowest in all tissues measured

The TPK protein expression levels in different organs were also studied. Because of inconsistent expression levels of internal reference proteins (β-actin and β-tubulin) across different tissues (Fig. 1e), the level of total protein obtained from Coomassie staining was used as the reference for TPK quantification (Fig. 1f). The results showed that the level of TPK protein in brain and heart tissue was the lowest among the examined tissues, followed by lung (2.2-fold higher), liver (3.2-fold higher), and highest in kidney (4.4-fold higher) compared with brain (Fig. 1g). The protein levels were in line with the *Tpk* mRNA levels, revealing that TPK mRNA and protein levels were notably lower in the brain and heart tissues than in other tissues assessed (Fig. 1a–g).

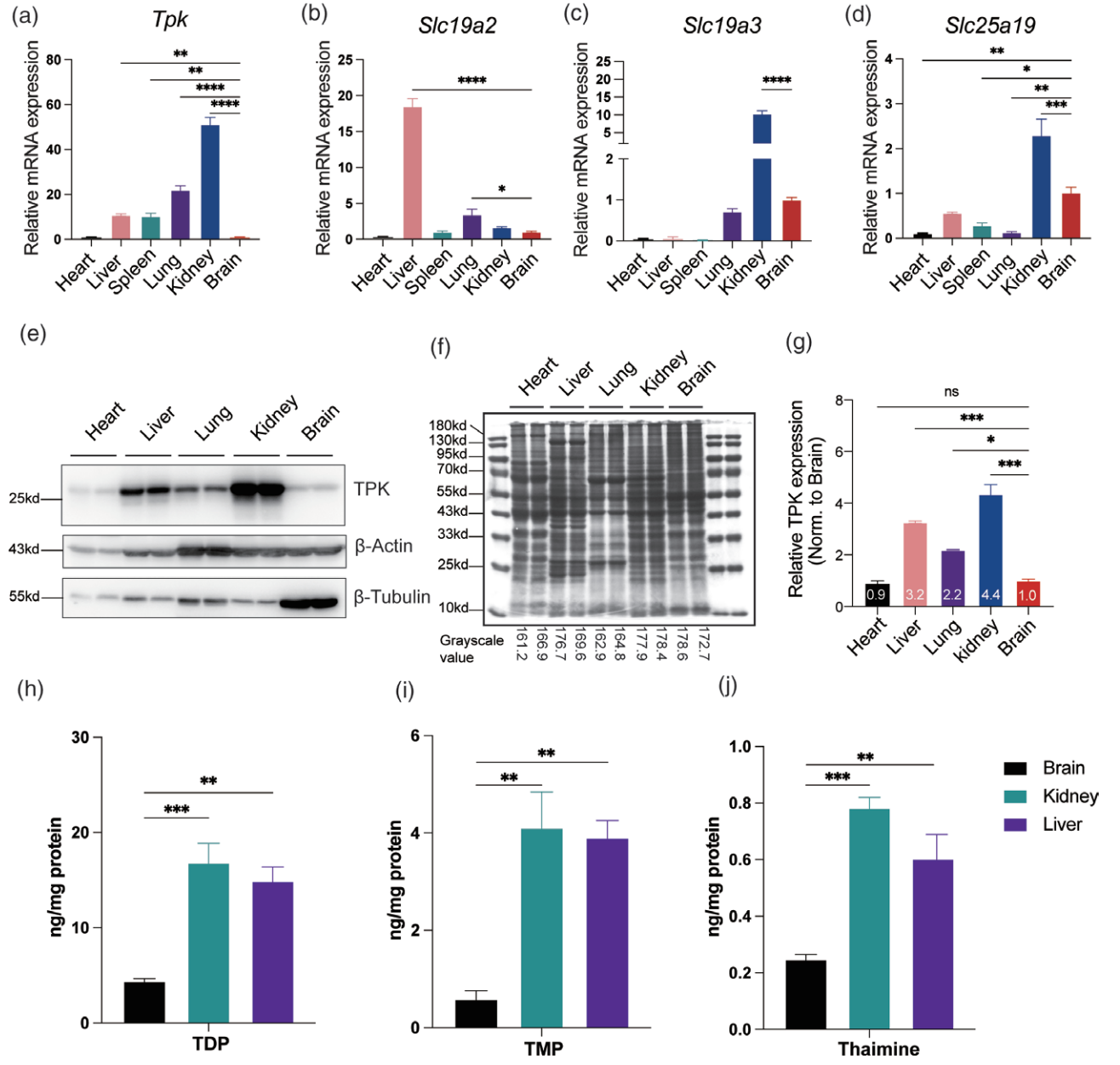
#### Levels of thiamine diphosphate, thiamine monophosphate, and thiamine in different tissues

The levels of TDP, TMP, and thiamine were measured in the brain, liver, and kidney of wild-type mice. The results showed that the levels of TDP, TMP, and thiamine were consistently higher in the kidney and liver compared to the brain, with the brain showing significantly lower levels (Fig. 1h–j). Overall, those results showed that the TPK expression level was the best indicator of thiamine metabolism status among the detected thiamine metabolism genes.

#### Pyrithiamine significantly reduces thiamine diphosphate levels in the brain

Pyrithiamine is a specific inhibitor of TPK. Eleven days after pyrithiamine administration, the levels of TDP,

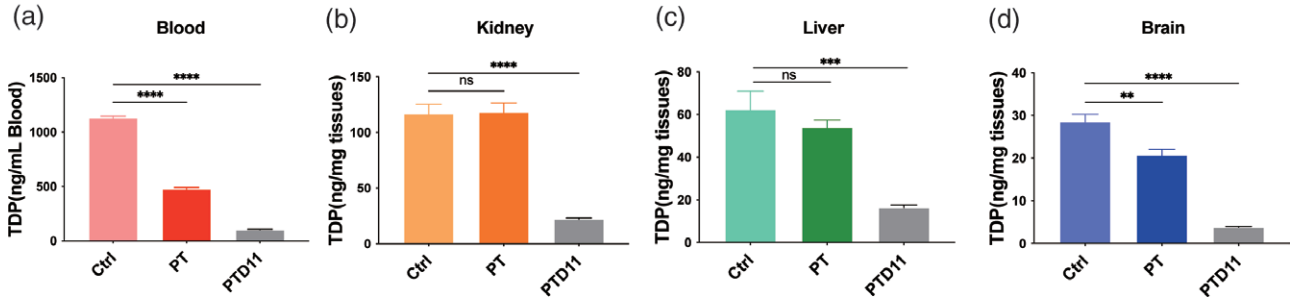
**Fig. 1**




Expression levels of *Tpk*, *Slc19a2*, *Slc19a3*, and *Slc25a19* in different tissues and tissue distribution of thiamine and phosphate esters. (a–d) Relative mRNA expression for the *Tpk* (a), *Slc19a2* (b), *Slc19a3* (c), and *Slc25a19* (d) in heart, liver, spleen, lung, kidney, and brain tissues of wild-type mice. Each gene expression was normalized to *Actb* ($N = 4$). (e) Western blot images for the TPK, β-tubulin, and β-actin protein expression levels in heart, liver, lung, kidney, and brain tissues of wild-type mice. (f) SDS-PAGE gel stained with Coomassie Brilliant Blue, with the grayscale values of each lane indicated below. (g) Quantification of TPK protein expression level, obtaining relative TPK expression for each tissue by correcting with total protein from Coomassie staining and then normalized to TPK value of brain ($N = 4$). (h–j) The TDP (h), TMP (i), and thiamine (j) content in the brain, kidney, and liver tissues of wild-type mice. The content is corrected by tissue weight. $N = 4$ for all groups. Data were expressed as mean ± SEM. One-way ANOVA with Tukey's post hoc test, and two-sided were used for comparisons; all groups compared with the brain, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. TDP, thiamine diphosphate; TPK, thiamine pyrophosphokinase-1; TMP, thiamine monophosphate.

TMP, and thiamine in the blood, the kidney, the brain, and the liver were investigated. TDP levels in the blood and the brain were significantly decreased as compared with that in control mice (Fig. 2a and d), TDP levels were not significantly changed in the kidney and the liver (Fig. 2b and c). While, the levels of TMP and thiamine all significantly decreased in the blood, kidney, brain, and liver, TMP was the greatest decline in the brain and thiamine was the greatest decline in the liver (Table 1). This may be due to pyrithiamine blocking TMP and thiamine protein binding sites, making nonprotein-binding thiamine more vulnerable to being lost. Therefore, our data

**Fig. 2**




Pyrithiamine significantly reduces TDP levels in the brain. (a–d) TDP levels during pyrithiamine treatment for 11 days (PT) or thiamine-deficient diet combined with PT treatment for 11 days (PTD11), and control mice (Ctrl) in the blood (a), kidney (b), liver (c), and brain (d) ($N = 4$ for all groups). The data were expressed as mean ± SEM. One-way ANOVA with Tukey's post hoc test, and two-sided were used for comparisons. ns indicates nonsignificant differences; $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. TDP, thiamine diphosphate.

**Table 1 The concentration of thiamine monophosphate (TMP) and thiamine in different tissues after PT treatment or PTD treatment**

| | TMP (ng/mg (mL)) | | | Thiamine (ng/mg (mL)) | | |
|---|---|---|---|---|---|---|
| Tissue | Ctrl ($N = 4$) | PT ($N = 4$) | PTD ($N = 4$) | Ctrl ($N = 4$) | PT ($N = 4$) | PTD ($N = 4$) |
| Blood | 93.73 ± 3.25 | 70.85 ± 1.43****a | 4.67 ± 1.27####b | 119.40 ± 2.23 | 53.85 ± 1.59****a | 7.01 ± 1.13####b |
| Kidney | 55.61 ± 5.82 | 2.59 ± 0.16***a | 4.13 ± 0.51####b | 6.21 ± 0.72 | 26.16 ± 2.38***a | 0.28 ± 0.04####b |
| Liver | 75.40 ± 3.00 | 5.21 ± 0.38***a | 3.33 ± 0.64####b | 17.07 ± 3.09 | 30.96 ± 1.64****a | 0.54 ± 0.12####b |
| Brain | 10.45 ± 1.68 | 1.26 ± 0.11****a | 0.37 ± 0.05####b | 2.19 ± 0.02 | 3.40 ± 0.41***a | 0.06 ± 0.01####b |

Data were expressed as mean ± SEM; Student's *t*-test were used for comparisons. 'a' means PT group compared with Ctrl group; 'b' means PTD group compared with Ctrl group.

PT, pyrithiamine; PTD, thiamine deficiency-diet combined with PT treatment for 11 days.

***$P < 0.001$.

****$P < 0.0001$.

##

##$P < 0.0001$.

provided a molecular rationale for TPK in determining bodily thiamine metabolism and its lowest level in the brain and heart tissues underlying the tissue sensitivity due to thiamine deficiency.

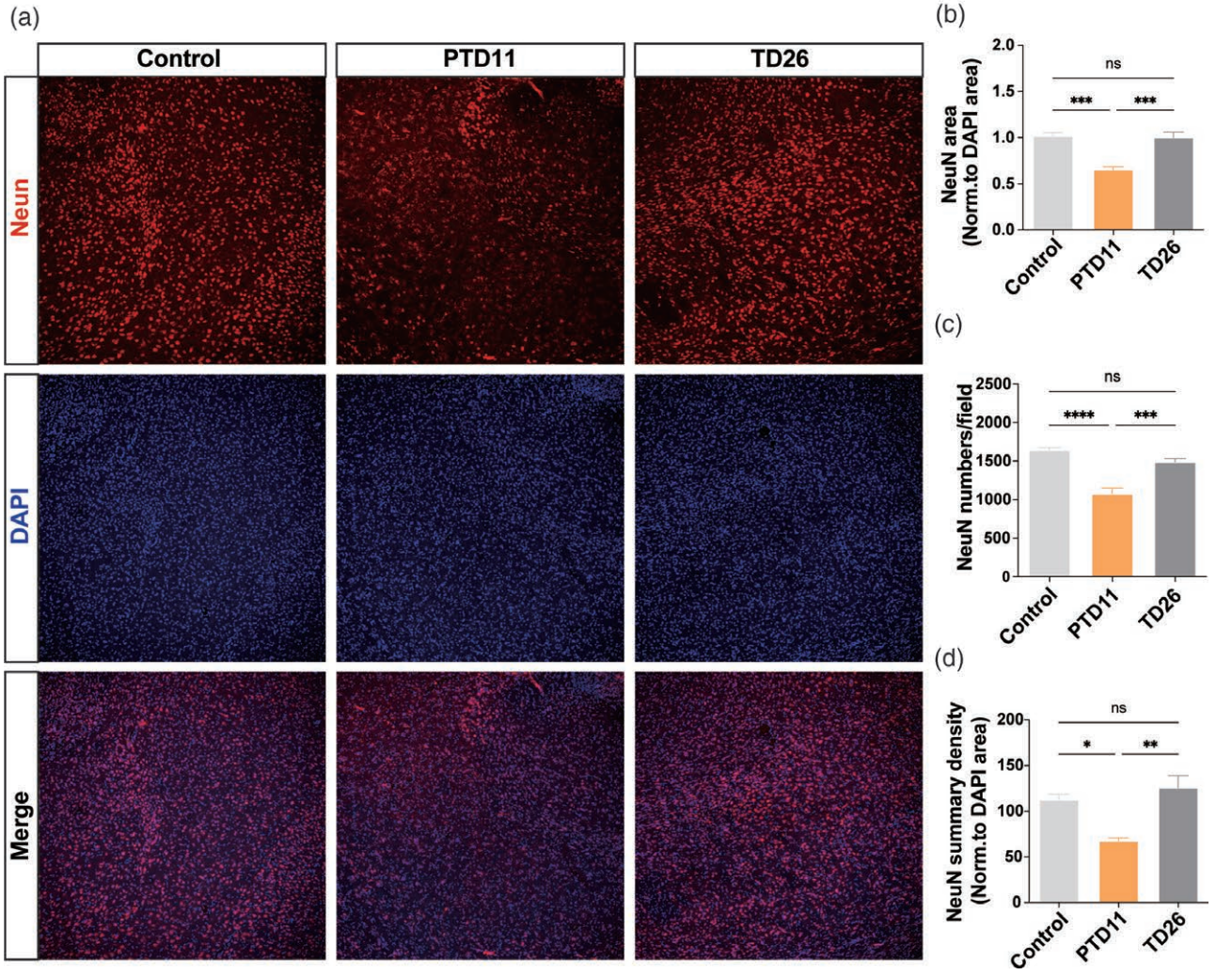
In order to further determine whether pyrithiamine combined with thiamine deficiency (PTD) can further lead to thiamine metabolism inhibition, we measured the levels of TDP, TMP, and thiamine in mice with PTD on the 11$^{th}$ day (PTD11), and the results showed that TDP were significantly reduced in the blood, liver, kidney and brain of PTD mice. More importantly, PTD can significantly reduce the level of TDP in the brain compared with pyrithiamine administration alone (Fig. 2a–d). The levels of TMP and thiamine were also significantly reduced in various tissues of PTD mice (Table 1).

### Thiamine pyrophosphokinase-1 inhibition significantly induced neuron loss, neuroinflammation, and blood-brain barrier broken

The most typical thiamine deficiency-induced brain pathological lesions include neuron loss, astrocyte and microglia activation, and BBB damage [18,20,21]. To clarify the effect of TPK inhibition on brain injury in mice, we performed histological analysis on brain slices from PTD, TD, and control mice. Firstly, when the NeuN antibody was used to stain the brain slices to detect neuronal damage, the results showed that the number of NeuN-positive cells and the fluorescence intensity in the PTD mice were significantly reduced compared to the control mice (Fig. 3a–d). Secondly, staining brain glia cells with the microglia marker IBA1 and the astrocyte marker GFAP antibodies to demonstrate the activation of inflammation showed that both the number and intensity of IBA1-positive cells (Fig. 4a–d) and GFAP-positive cells (Fig. 4e–h) were significantly higher than those in the control group. Finally, when antibodies to IgG and lectin were used to stain brain slices, the results showed that in PTD mice IgG leakage was increased, suggesting destruction of the BBB, and lectin labeling of blood vessels showed increased vascularization and structural disorder (Fig. 5a–d). All these results show that pyrithiamine combined with thiamine-deficient diet can induce significant neuron loss, neuroinflammation, broken BBB, and vascular damage compared with control mice.

After feeding the thiamine-depleted diet for 26 days, the TD26 mice showed significant body weight loss

**Fig. 3**




TPK inhibition significantly induced neuron loss. (a) Representative images of neurons stained with NeuN (red) and counterstained with DAPI (blue) in thalamus of control, PTD11, and TD26 mice. (b–d) Quantification of NeuN-immunoreactivity neurons covered summary areas (b), numbers (c), and summary density (d) in thalamus of control, PTD11, and TD26 mice ($N = 4$ for all groups). Data were expressed as mean ± SEM. One-way ANOVA with Tukey's post hoc test, and two-sided were used for comparisons. ns indicates nonsignificant differences; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. Scale bars: 50 μm. PTD11, thiamine-deficient diet combined with PT treatment for 11 days; TD26, thiamine-deficient diet for 26 days. TPK, thiamine pyrophosphokinase-1.

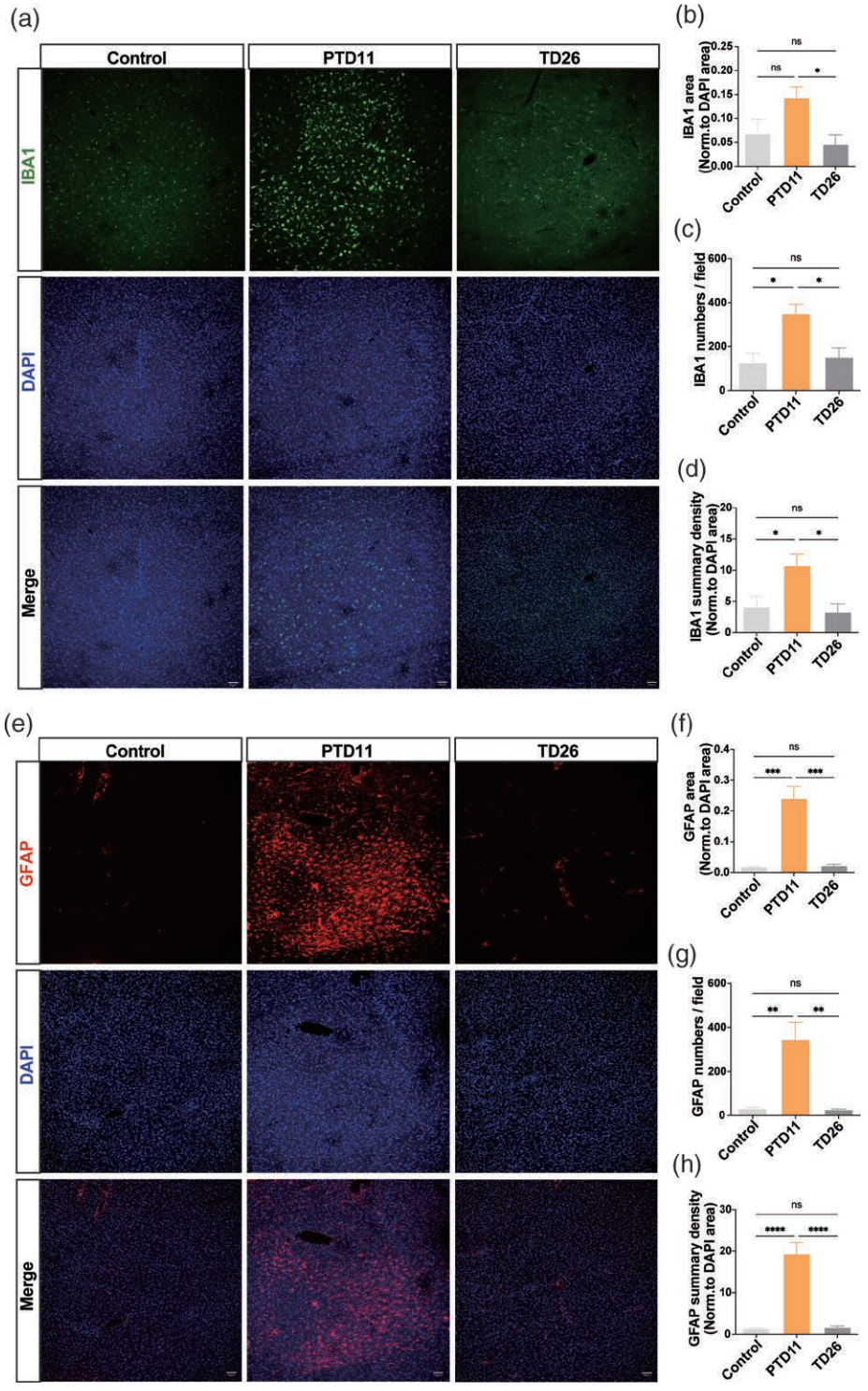
and were near death compared with control mice. When pathologic brain damage in PTD mice and TD mice were compared, we found that there was less neuron loss in TD26 mice compared with PTD11 mice, where it was comparable to that in the control group (Fig. 3a–d). Activation and proliferation of astrocytes and microglia were also significantly decreased in TD26 mice compared with PTD11 mice, and not significantly changed compared with control mice (Fig. 4a–h). Vascular damage and IgG leakage observed in PTD11 mice were also comparable to that seen in the control mice (Fig. 5a–d).

Collectively, those results indicated that injection of a specific TPK inhibitor pyrithiamine can significantly inhibit the production of TDP in brain tissue, leading to neuronal loss and an inflammation-activated phenotype. This effect of a TPK inhibitor further demonstrated the important role of TPK in thiamine deficiency-induced brain susceptibility.

## Discussion

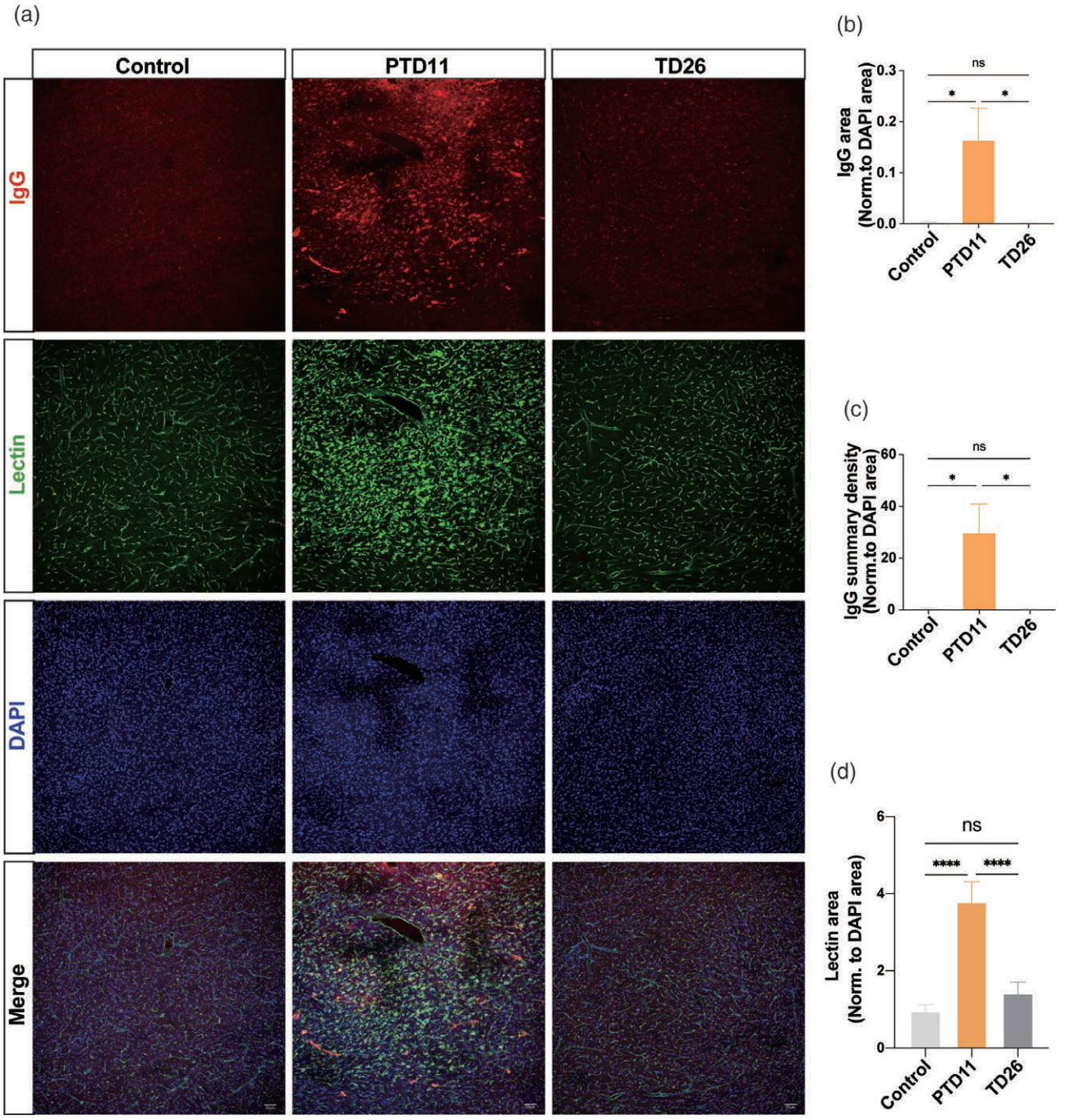
These results demonstrate that the brain exhibits a lower TPK expression than that in the liver and kidney of wild-type mice in this study. Among the detected thiamine metabolism genes, TPK expression level was the best indicator of thiamine metabolism status in the different tissues. Pyrithiamine, a specific TPK inhibitor, no matter whether administered alone (pyrithiamine) or as part of a thiamine-deficient diet (PTD), consistently significantly

**Fig. 4**




TPK inhibition significantly induced neuroinflammation. (a) Representative images of microglia stained with IBA1 (green) and counterstained with DAPI (blue) in thalamus of control (Ctrl), PTD11, and TD26 mice. (b–d) Quantification of IBA1-immunoreactive microglia showing summary areas (b), numbers (c), and summary density (d) in thalamus of control, PTD11, and TD26 mice ($N = 4$ for all groups). (e) Representative images of astrocytes stained with GFAP (red) and counterstained with DAPI (blue) in thalamus of control, PTD11, and TD26 mice. (f–h) Quantification of GFAP-immunoreactive astrocytes showing summary areas (f), numbers (g), and summary density (h) in thalamus of control, PTD11, and TD26 mice ($N = 4$ for all groups). Data were expressed as mean ± SEM. One-way ANOVA with Tukey's post hoc test, and two-sided were used for comparisons. ns indicates nonsignificant differences; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. Scale bars: 50 μm. PTD11, thiamine-deficient diet combined with PT treatment for 11 days; TD26, thiamine-deficient diet for 26 days; TPK, thiamine pyrophosphokinase-1.

**Fig. 5**




TPK inhibition significantly induced vascular leakage. (a) Representative images of IgG staining (red) and counterstained with DAPI (blue) in thalamus regions of control, PTD11, and TD26 mice. (b–d) Quantification of IgG-immunoreactivity covered summary areas (b), summary density (c), and lectin summary areas (d) in thalamus regions of control, PTD11, and TD26 mice ($N = 4$ for all groups). Data were expressed as mean ± SEM. One-way ANOVA with Tukey's post hoc test, and two-sided were used for comparisons. ns indicates nonsignificant differences; $^{*}P < 0.05$ and $^{****}P < 0.0001$. Scale bars: 50 μm. PTD11, thiamine-deficient diet combined with PT treatment for 11 days; TD26, thiamine-deficient diet for 26 days; TPK, thiamine pyrophosphokinase-1.

disrupted brain thiamine metabolism. Functionally, inhibited TPK combined with a thiamine-deficient diet induced brain lesions including neuron loss, neuroinflammation, and BBB permeability, whereas a thiamine-deficient diet alone did not. Our results necessarily and sufficiently demonstrated that the low TPK expression in the brain contributed to thiamine deficiency-induced brain susceptibility.

It has been well reported that thiamine deficiency is prone to induce severe brain disorders including dry beriberi, Wernicke's encephalopathy, and Wernicke–Korsakoff Syndrome [17,22]. The neuropathological changes observed in WKS are lesions in periventricular regions and typical atrophy of the mamillary bodies [23]. In addition to the mamillary body atrophy, WKS also involves neuronal loss in the anterior principal and medio-dorsal nuclei of the thalamus and the basal forebrain [17]. In animal studies, only the brain pathologies and behavioral symptoms of pyrithiamine-induced thiamine deficiency rodent models have been well-mapped to specific pathological changes in the anatomy and neurochemistry of human patients with thiamine deficiency. In our study, we found that the pyrithiamine-induced thiamine deficiency mice model also exhibited significant thiamine metabolism disorder and ultimately neuronal loss, bleeding, and inflammation-activated phenotype in the thalamus, which was limited in the brain of the single thiamine-deficient diet treated mice.

The above-mentioned two experimental rodent models of thiamine deficiency have been widely used. The first model was induced by simple thiamine-deficient diet food feeding to rodents for continuous 4 weeks in mice, and 6–7 weeks in rats. The second was more acutely induced by combining the feeding of a thiamine-free diet with the administration of a thiamine pyrophosphokinase inhibitor, pyrithiamine, termed pyrithiamine induced thiamine deficiency (PTD). The PTD model is a well-established experimental model that recapitulates the stereotyped progression of neurological/behavioral symptoms that mimic the pathology described in humans with TD [21]. The underlying mechanism by which the two models differ remains a mystery. Here our results demonstrated that the brain's low TPK contributed to thiamine deficiency-induced brain susceptibility. Thiamine-deficiency induced encephalopathy in humans is usually caused by simple thiamine deficiency, unlike the PTD mice model. The different structures and modifications of TPK between humans and mice also need further to be clarified.

Previous studies have tried to explain the thiamine deficiency-induced brain susceptibility from the perspective that the brain may be more prone to thiamine loss. However, the brain and heart exhibited more TDP conservation than other tissues after thiamine-deficient diet treatment [8]. Those results do not support this hypothesis. Our study is the first to explore whether the tissue distribution of different thiamine metabolism genes may mediate tissue sensitivity to thiamine deficiency. We found only TPK was the consistently lower expression in thiamine deficiency susceptive tissues: brain and heart. Combined with pharmacological experimental results using pyrithiamine, it necessarily and sufficiently demonstrated that the low TPK expression in the brain contributed to thiamine deficiency-induced brain susceptibility.

We also compared the thiamine metabolism genes with thiamine and its ester levels and found the TPK expression levels were most closely correlated with TDP, TMP, and thiamine levels. Rapala-Kozik *et al*. reported that TPK and TDP phosphatases coordinately regulated and stabilized the pool of TDP in Zea maize seedlings or under abiotic stress [24,25]. Those results suggested that TPK-regulated TDP homeostasis is conserved between mammals and plants.

Our study also found that the TPK level in heart tissue is as low as in the brain, leading us to speculate that low expression of TPK may be a significant factor contributing to the heart's susceptibility to thiamine deficiency. Multiple studies have found that thiamine deficiency not only leads to neurological abnormalities but also plays a crucial role in the process of high output heart failure, known as wet beriberi. Heart failure caused by thiamine deficiency is characterized by cardiac enlargement with a normal rhythm, dependent edema, elevated venous pressure, peripheral neuritis or pellagra, and nonspecific electrocardiogram changes. This condition typically follows a severe dietary deficiency of thiamine lasting 3 months or more, but symptoms often improve with vitamin supplementation [26]. In further studies we will tissue-specifically overexpress TPK in order to explore this issue.

### Conclusion

In summary, we elucidated that TPK expression level was the best indicator of thiamine metabolism status. The difference in TPK expression among various tissues may contribute to tissue susceptibility induced by thiamine deficiency. This study increased our understanding of the molecular basis of thiamine metabolism in different tissues and the clinical manifestations caused by thiamine deficiency. TPK will be a new therapy target for disrupted TDP homeostasis-related neurological disease.

## Acknowledgements

This study was supported by grants from the Shanghai Municipal Science and Technology Major Project, the National Natural Science Foundation of China (81870822, 91332201, 81901081, 81600930, 81500907), and the Natural Science Foundation of Fujian Province (2020CXB049).

### Conflicts of interest

Chunjiu Zhong, one of the corresponding authors, holds shares of Shanghai Raising Pharmaceutical Co., Ltd., which is dedicated to developing drugs for the prevention and treatment of AD. For the remaining authors, there are no conflicts of interest

## References

1 Manzetti S, Zhang J, van der Spoel D. Thiamin function, metabolism, uptake, and transport. *Biochemistry* 2014; **53**:821–835.
2 Haas RH. Thiamin and the brain. *Annu Rev Nutr* 1988; **8**:483–515.

3 Gibson GE, Hirsch JA, Fonzetti P, Jordan BD, Cirio RT, Elder J. Vitamin B1 (thiamine) and dementia. *Ann N Y Acad Sci* 2016; **1367**:21–30.
4 Ridley NJ, Draper B, Withall A. Alcohol-related dementia: an update of the evidence. *Alzheimers Res Ther* 2013; **5**:3.
5 Sang S, Pan X, Chen Z, Zeng F, Pan S, Liu H, *et al*. Thiamine diphosphate reduction strongly correlates with brain glucose hypometabolism in Alzheimer's disease, whereas amyloid deposition does not. *Alzheimers Res Ther* 2018; **10**:26.
6 Pan X, Sang S, Fei G, Jin L, Liu H, Wang Z, *et al*. Enhanced activities of blood thiamine diphosphatase and monophosphatase in Alzheimer's disease. *PLoS One* 2017; **12**:e0167273.
7 Pan X, Fei G, Lu J, Jin L, Pan S, Chen Z, *et al*. Measurement of blood thiamine metabolites for Alzheimer's disease diagnosis. *EBioMedicine* 2016; **3**:155–162.
8 Klooster A, Larkin JR, Wiersema-Buist J, Gans RO, Thornalley PJ, Navis G, *et al*. Are brain and heart tissue prone to the development of thiamine deficiency? *Alcohol* 2013; **47**:215–221.
9 DREYFUS PM. The quantitative histochemical distribution of thiamine in deficient rat brain. *J Neurochem* 1961; **8**:139–145.
10 Rindi G, Patrini C, Laforenza U, Mandel H, Berant M, Viana MB, *et al*. Further studies on erythrocyte thiamin transport and phosphorylation in seven patients with thiamin-responsive megaloblastic anaemia. *J Inherit Metab Dis* 1994; **17**:667–677.
11 Ortigoza-Escobar JD, Molero-Luis M, Arias A, Marti-Sanchez L, Rodriguez-Pombo P, Artuch R, Pérez-Dueñas B. Treatment of genetic defects of thiamine transport and metabolism. *Expert Rev Neurother* 2016; **16**:755–763.
12 Kono S, Miyajima H, Yoshida K, Togawa A, Shirakawa K, Suzuki H. Mutations in a thiamine-transporter gene and Wernicke's-like encephalopathy. *N Engl J Med* 2009; **360**:1792–1794.
13 Labay V, Raz T, Baron D, Mandel H, Williams H, Barrett T, *et al*. Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness. *Nat Genet* 1999; **22**:300–304.
14 Mayr JA, Freisinger P, Schlachter K, Rolinski B, Zimmermann FA, Scheffner T, *et al*. Thiamine pyrophosphokinase deficiency in encephalopathic children with defects in the pyruvate oxidation pathway. *Am J Hum Genet* 2011; **89**:806–812.
15 Banka S, de Goede C, Yue WW, Morris AAM, von Bremen B, Chandler KE, *et al*. Expanding the clinical and molecular spectrum of thiamine pyrophosphokinase deficiency: a treatable neurological disorder caused by TPK1 mutations. *Mol Genet Metab* 2014; **113**:301–306.
16 Biesecker LG. Amish lethal microcephaly. *Am J Med Genet*1993; **52**:598–603.
17 Zahr NM, Kaufman KL, Harper CG. Clinical and pathological features of alcohol-related brain damage. *Nat Rev Neurol* 2011; **7**:284–294.
18 Horita N, Okuno A, Izumiyama Y. Neuropathologic changes in suckling and weanling rats with pyrithiamine-induced thiamine deficiency. *Acta Neuropathol* 1983; **61**:27–35.
19 Sanemori H, Kawasaki T. Purification and properties of thiamine pyrophosphokinase in *Paracoccus denitrificans*. *J Biochem* 1980; **88**:223–230.
20 Mouton-Liger F, Rebillat AS, Gourmaud S, Paquet C, Leguen A, Dumurgier J, *et al*. PKR downregulation prevents neurodegeneration and beta-amyloid production in a thiamine-deficient model. *Cell Death Dis* 2015; **6**:e1594.
21 Vetreno RP, Ramos RL, Anzalone S, Savage LM. Brain and behavioral pathology in an animal model of Wernicke's encephalopathy and Wernicke-Korsakoff syndrome. *Brain Res* 2012; **1436**:178–192.
22 Stroh C, Meyer F, Manger T. Beriberi, a severe complication after metabolic surgery – review of the literature. *Obes Facts* 2014; **7**:246–252.
23 Arts N, Pitel AL, Kessels R. The contribution of mamillary body damage to Wernicke's encephalopathy and Korsakoff's syndrome. *Handb Clin Neurol* 2021; **180**:455–475.
24 Rapala-Kozik M, Golda A, Kujda M. Enzymes that control the thiamine diphosphate pool in plant tissues. Properties of thiamine pyrophosphokinase and thiamine-(di)phosphate phosphatase purified from *Zea mays* seedlings. *Plant Physiol Biochem* 2009; **47**:237–242.
25 Rapala-Kozik M, Kowalska E, Ostrowska K. Modulation of thiamine metabolism in *Zea mays* seedlings under conditions of abiotic stress. *J Exp Bot* 2008; **59**:4133–4143.
26 Roman-Campos D, Cruz JS. Current aspects of thiamine deficiency on heart function. *Life Sci* 2014; **98**:1–5.

# Essential Titles in RADIOLOGY


Search articles, register for eAlerts, view abstracts, and SUBSCRIBE online at **journals.lww.com**.

Wolters Kluwer

8-R172