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Intestinal Piezo1 Promotes Neuroinflammation to Facilitate Oligodendrocyte Ferroptosis Post-Traumatic Brain Injury

(Usus Piezo1 Menggalakkan Keradangan Neuro untuk Memudahkan Oligodendrocyte Ferroptosis Kecederaan Otak Pasca Traumatik)

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ABSTRACT

The coexistence of traumatic brain injury (TBI) and cognitive impairment is increasingly common in clinical practice, but current research on identifying biomarkers for such conditions and precise intervention points is insufficient. This study utilized bioinformatics analysis with paired samples to explore potential causal links between inflammatory factors and TBI and cognitive impairment. By constructing a TBI model with Piezo1 gene knockout, we assessed the activation status of microglia in the brain, the differentiation process of oligodendrocyte precursor cells. The study identified a series of inflammatory factors significantly associated with TBI, including C-C motif chemokine 19, C-X-C motif chemokine 5, and interleukin-5. Bioinformatics analysis showed increased expression of CXCL10, CCL2, GNA15, NFKB1, and the top 5 key nodes were identified using the Cytohubba plugin. The experimental results indicated that the knockout of the Piezo1 gene significantly reduced the infiltration of microglia and neuroinflammation in the brain.

Keywords: Bioinformatics; CXCL10; neuroinflammation; traumatic brain injury

ABSTRAK

Kewujudan kecederaan otak traumatis (TBI) dan gangguan kognitif semakin menjadi kebiasaan dalam amalan klinikal, tetapi penyelidikan semasa mengenai pengenalpastian penanda biologi untuk keadaan sedemikian dan titik intervensi yang tepat adalah tidak mencukupi. Kajian ini menggunakan analisis bioinformatik dan randomisasi Mendelian (MR) dengan sampel bersama untuk meneroka hubungan kausal berpotensi antara faktor inflamasi dengan TBI dan gangguan kognitif. Dengan membina model TBI dengan penutupan gen Piezo1, kami menilai status pengaktifan mikroglia dalam otak, proses pembezaan sel-sel pendahulu oligodendrosit. Kajian mengenal pasti siri faktor inflamasi yang signifikan berkaitan dengan TBI, termasuk kemokina motif C-C 19, kemokina motif C-X-C 5 dan interleukin-5. Analisis bioinformatik menunjukkan peningkatan ekspresi CXCL10, CCL2, GNA15, NFKB1 dan 5 nod kunci teratas dikenal pasti menggunakan plugin Cytohubba. Keputusan uji kaji menunjukkan bahawa penutupan gen Piezo1 secara signifikan mengurangkan infiltrasi mikroglia dalam otak dan melindungi pembezaan oligodendrosit.

Kata kunci: Bioinformatik; CXCL10; kecederaan otak traumatis; keradangan neuro

INTRODUCTION

Traumatic brain injury (TBI) is a global health issue that often leads to long-term cognitive impairment. The inflammation mediated by microglia cells plays a crucial role in the development of neurological dysfunction following TBI (Hiwase et al. 2024; Metry et al. 2024; Rapport et al. 2024). Modulation of the inflammatory microenvironment caused by microglia cells could be a useful therapeutic strategy for alleviating cognitive deficits associated with TBI. Microglia are the resident immune cells of the CNS and have essential roles in brain homeostasis as well as under pathological conditions (Chen et al. 2024b; Fujisawa et al. 2024). The central role of astrocytes in neuroinflammation has been highlighted and it is widely accepted that dysregulated activation/blockade of microglia cells implicated the initiation/elimination, respectively, have an impact on various neurological diseases. This perspective should provide

exact therapeutic strategies instead of mere amelioration the neuroinflammatory responses by targeting microglial cells. Strategically managing the rise of microglia cells, fine-tuning between neuroprotection and damage may be an emerging therapeutic targets for diseases accompanied by excessive activation or senescence-associated reduced immune responses in brain tissue. More work is needed to understand the precise signaling pathways modulating microglial activation and neuroinflammatory activities which will provide a foundation for novel therapeutic approaches.

A growing body of research indicates that gut function plays a crucial role in human health. It not only has an important role in immune system and intestinal homeostasis of the host, but also contributes to metabolism of drugs. This is especially true as it relates to diseases of metabolism and neurodegenerative conditions, heavily influenced by the gut microbiota (Ma et al. 2024; Rajkumar 2024). This phenomenon has been associated with a variety of pathologies including neuroinflammation, defective immune function in the gut or dysbiotic imbalance leading to pro-inflammatory pathways and ultimately inducing further diseases ranging from systemic disorders to cognitive deterioration due brain accumulation amyloid-beta/tau aggregates plus increased permeability of BBB that pave way for other events triggering Cognitive Impairment.

Inflammatory factors are one of the most vital reasons related to cognitive deficit post TBI (Minea et al. 2024; Zhang et al. 2024). Inflammation in the injured brain after TBI affects not only local injury but also systemic cytokines, influencing cognitive function. Literature has shown that TBI can result in neural damage associated with cognitive decline being mediated by several inflammatory factors related to cytokines and chemokine responses. These inflammatory factors destroy brain cells, damage the connectivity of neurons and reduce processes for neural regeneration which all have a negative effect on cognitive function.

In addition, the blood-brain barrier may also be damaged post-TBI which would result in enhanced inflammatory factor levels and heightened neuroinflammation-induced cognitive impairment. Thus, the suppression of inflammatory response after TBI may be able to reduce cognitive deficits in humans even if inflammation is mild and occurs at an early stage. Therefore, our results indicate that targeting the interaction between target cells and inflammatory factors is a new sight to understand as well as possibly treat cognitive deficits after TBI. By further exploring these pathways, we may gain a useful perspective on the pathophysiology of TBI-induced cognitive deficits and design more efficient interventions.

MATERIALS AND METHODS

DIFFERENTIAL GENE ANALYSIS FOR GSE86579

GSE86579 contains 5 normal samples and 6 tbi samples, with 200 inflammation-related genes from hallmark inflammatory response.

GO/KEGG ANALYSIS

We utilized the ‘clusterProfiler’ R package to conduct Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, aiming to decipher the intrinsic mechanisms driving disease progression and etiology, when using ‘clusterProfiler’ for analysis, you can refer to its official documentation and tutorials to ensure the correct usage of these parameters. More detailed information and parameter settings can be found on the RDocumentation and Bioconductor pages of ‘clusterProfiler’ (Dvorak et al. 2024; Xu et al. 2024).

PROTEIN-PROTEIN INTERACTION NETWORK

We deployed the STRING2 and Cytoscape software suite for the anticipation and graphical representation of molecular interactions, as well as the construction of protein-protein interaction (PPI) networks (Hayashi et al. 2024; Oduro-Kwateng et al. 2024). Within Cytoscape, the degree algorithm was engaged to prioritize significant genes within the PPI framework. Furthermore, we harnessed the Networkanalyst online portal to prognosticate the microRNAs and transcription factors associated with the pivotal genes.

ANIMALS AND EXPERIMENTAL DESIGN

Aged six to eight weeks, C57BL/6 wild-type (WT) mice were procured from the Shanghai Slake Laboratory Animal Company. The provision of Villin-CreERT and Piezo1flox/flox mice was facilitated by the Shanghai Nanfang Mode Biotechnology Co., Ltd., with verification of their genotype accomplished through PCR analysis of tail-derived samples. The animals were housed in a pathogen-controlled environment, subjected to a 12-h alternating light and dark cycle at a temperature of 25 °C, and provided with unlimited access to food and water. Following a week-long period for environmental adaptation, the experimental procedures began. Mice were anesthetized by intraperitoneal injection of pentobarbital sodium at a dosage of 20 mg/kg body weight. Subsequently, they were randomly divided into four groups, each comprising four mice: A Control group (administered normal saline daily), a TBI group (subjected to a 4 mm craniotomy using a high-speed drill and a 2 mm bit to expose the cerebral cortex, then impacted with the

Impact One Stereotaxic Impactor at specified parameters: velocity of 4 m/s, dwell time of 200 ms, and depth of 1 mm), a TBI+Piezo1 KO group, and a Piezo1 KO group. The mice's brains were extracted 72 h post-treatment. This study was approved by the Animal Ethics Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, with an ethics number of 2021-028.

IMMUNOFLUORESCENCE

For immunofluorescence analysis of tissue samples, 20-micrometer-thick sections of brain tissue underwent deparaffinization and rehydration, followed by antigen retrieval. Subsequently, the renal tissue sections were incubated with 10% bovine serum albumin for one hour at ambient temperature to reduce non-specific binding. The sections were then exposed to a set of primary antibodies at 4 °C overnight, diluted at a ratio of 1:100, including: anti-IL-1β (Proteintech, catalog number 19771-1-AP), anti-IL-6 (AiFang, catalog number AF11476), anti-IL-1R (AiFang, catalog number AF14902).

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Enzyme-linked immunosorbent assay (ELISA) kits were utilized to measure the concentrations of IL-6, CXCL10, CXCL5, CCL2, and MMP9. The experimental procedure outlined in the respective reagent kit manual was followed, and the results were documented using an Excel spreadsheet.

STATISTICS

All statistical analyses were conducted with R software. The decision to employ a t-test or a Mann-Whitney U test was guided by the adherence of the data to a normal distribution pattern. A p-value of less than 0.05 was generally considered to indicate statistical significance.

RESULTS

DEG SELECTION

We first obtain the GSE86579 dataset from the GEO database and identify the DEGs of the TBI dataset. Compared with the normal group in the GSE86579 data set, the limma package was used for differential analysis, and it was found that 23 inflammation-related genes were differentially expressed between the two groups. Among them, CXCL10, CCL2, GNA15, NFKB1, CSF1, F3, NLRP3, CSF3R, CD82, P2RX7, ABCA1, TIMP1, SLC7A2, APLNR, GPR183, TAPBP, PDPN, EMP3, IRF1 were upregulated in TBI; while TACR1, KCNA3, ADRM1, KCNMB2 were downregulated in TBI (Figure 1(A)-1(D)).

GO/KEGG ANALYSIS

GO and KEGG analyzes were performed to further explore the potential roles of these 23 genes. GO enrichment analysis showed that core genes mainly affect biological functions such as leukocyte migration response to mechanical stimulus, response to molecule of bacterial origin. KEGG enrichment analysis shows that core genes mainly affect biological functions such as Pertussis, NOD−like receptor signaling pathway, Influenza A, and TNF signaling pathway (Figure 2(A) and 2(B)).

PPI NETWORK ANALYSIS

We used the STRING online tool to construct a PPI network of overlapping central genes (Figure 3(A)). Subsequently, the top five genes were visualized using Cytoscape software (Figure 3(B)). To put it simply, sort out TPX2, CCNB2, BUB1, TOP2A, and ASPM.

INTESTINAL PIEZO1 KNOCKOUT ALLEVIATES MICROGLIA-INDUCED NEUROINFLAMMATION FOLLOWING TBI

In comparison to the control group (Figure 4(A)-4(C)), both IL-1β and IL-6 protein expression levels was up-regulated in the the hippocampus region of brain tissue at 3 days after TBI injection (Figure 4(D)-4(L)). But Piezo1 KO administration blocked the expression of IL-1β and IL-6 (Figure 6(M)-6(X)). Results showed that the IL-1β immunoreactivities were induced in TBI-treated mice. However, piezo1 ko therapy reduced the expression level of IL-1β. In comparison to the control group, there was a significant increase in the protein expression level of IL-6 in the TBI group, and piezo1 ko treatment restored the expression of IL-6 (Figure 4(X)-4(Z)).

PIEZO1 KNOCKOUT ALLEVIATES EXPRESSION OF IL-1R ON OLIGODENDROCYTE FOLLOWING TBI

Comparatively, in the hippocampus region of the brain tissue, IL-1R protein expression levels were up-regulated at 3 days after TBI injection when compared to the control group (Figure 5(A)-5(M)). However, administration of piezo1 ko inhibited the expression of IL-1R. Compared with the control group, the expression of IL-1β and IL-1RmRNA is increased, as shown in Figure 5(N) and 5(O).

PIEZO1 KNOCKOUT INHIBITS M1 ACTIVATION FOLLOWING TBI

Elisa measured the expression of inflammatory factors secreted by M1-type microglial cells. Compared to the control group, the TBI group showed increased expression of CXCL10, CCL2, CXCL5, IL-5, IL-6, and MMP9. After intervention with piezo1 ko, the expression of CXCL10,

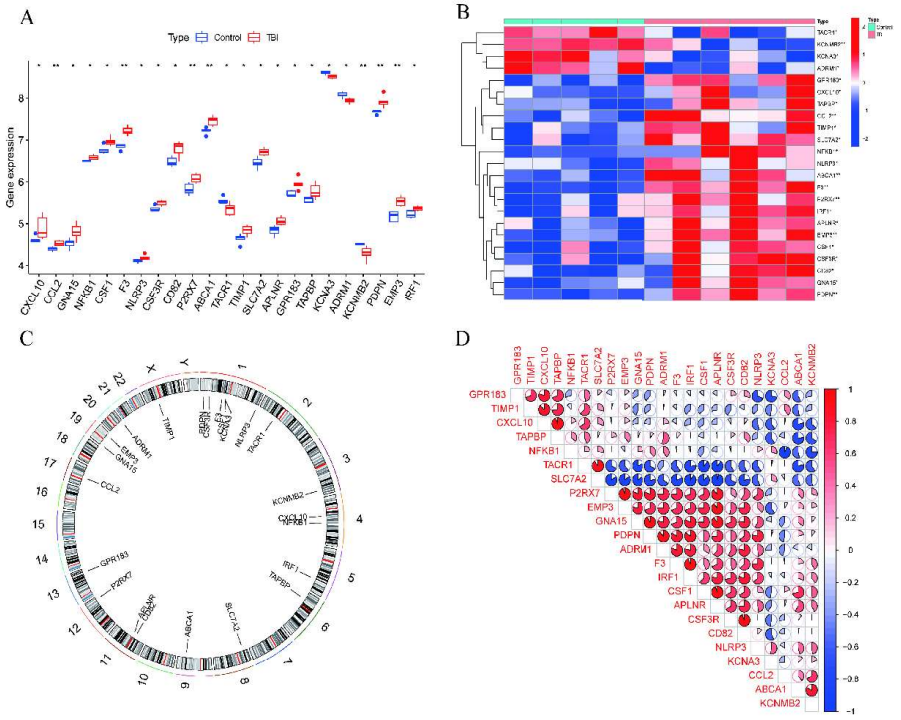
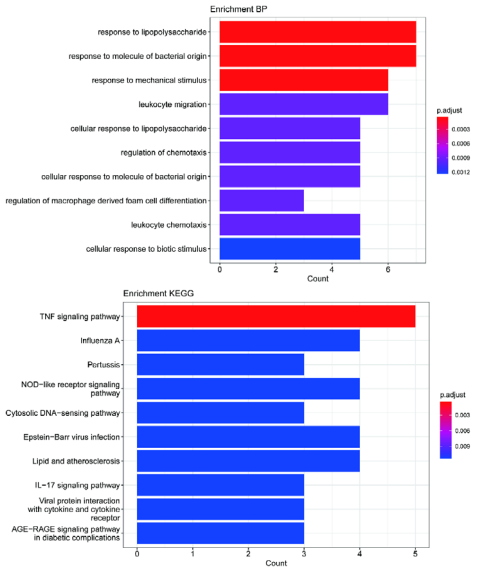


FIGURE 1. Analysis of differentially expressed inflammatory response genes (DE-IRGs) related to traumatic brain injury (TBI). A) Boxplot of differential expression of inflammatory response genes in TBI group vs. normal group; B) Heat map of DE-IRGs expression in TBI group vs. normal group; C) Chromosomal map of DE-IRGs; and D) Correlation heatmap of 23 DE-IRGs. * represents p<0.05; ** represents p<0.01



BP, Biological Process; KEGG, Kyoto Encyclopedia of Genes and Genomes

FIGURE 2. Functional enrichment analysis of differentially expressed inflammatory response genes (DE-IRGs). A) The BP functional enrichment of DE-IRGs; B) The KEGG analysis of DE-IRGs

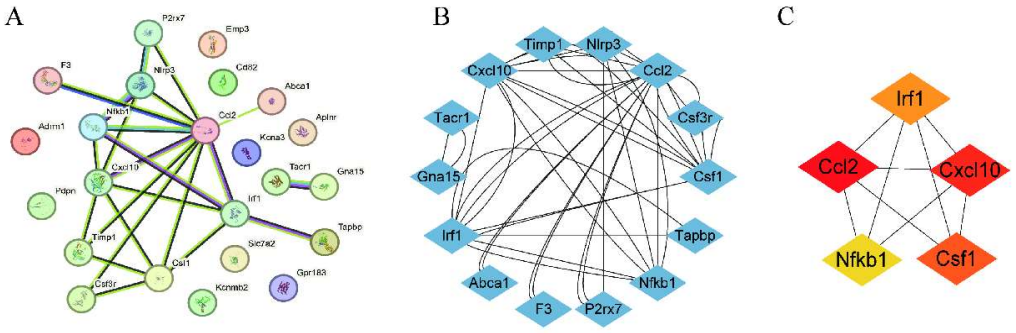


FIGURE 3. Construction of protein-protein interaction (PPI) network. A) PPI network constructed by differentially expressed inflammatory response genes (DE-IRGs) using STRING database; B) Cytoscape network; and C) Top five cytohubba-MCC hub genes

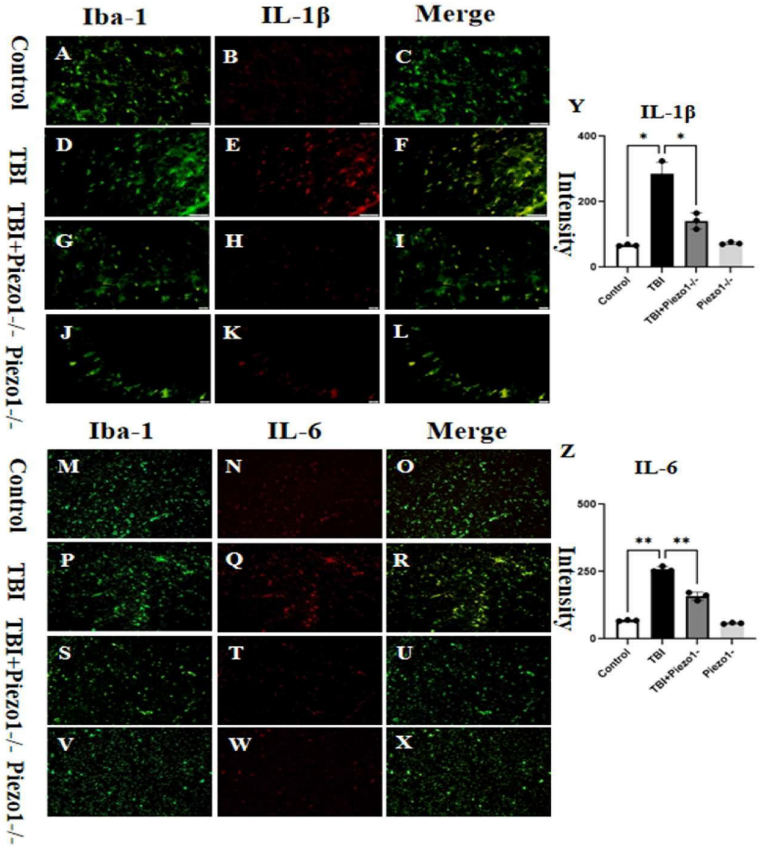


FIGURE 4. Piezo1 knockout alleviates microglia-induced neuroinflammation following TBI. Compared to the control group (A-C), the expression levels of both IL-1β and IL-6 proteins were up-regulated in the hippocampal region of brain tissue at 3 days after TBI injection (D-L). However, administration of Piezo1 KO blocked the expression of IL-1β and IL-6 (M-X). The results indicated that TBI-treated mice exhibited induced IL-1β immunoreactivities, whereas Piezo1 KO therapy reduced the expression level of IL-8. Furthermore, the protein expression level of IL-6 in the TBI group showed a significant increase compared to the control group, but Piezo1 KO treatment restored the expression of IL-6 (X-Z).*P <0.05,**P <0.01, Scale bars: A–X 50 μm

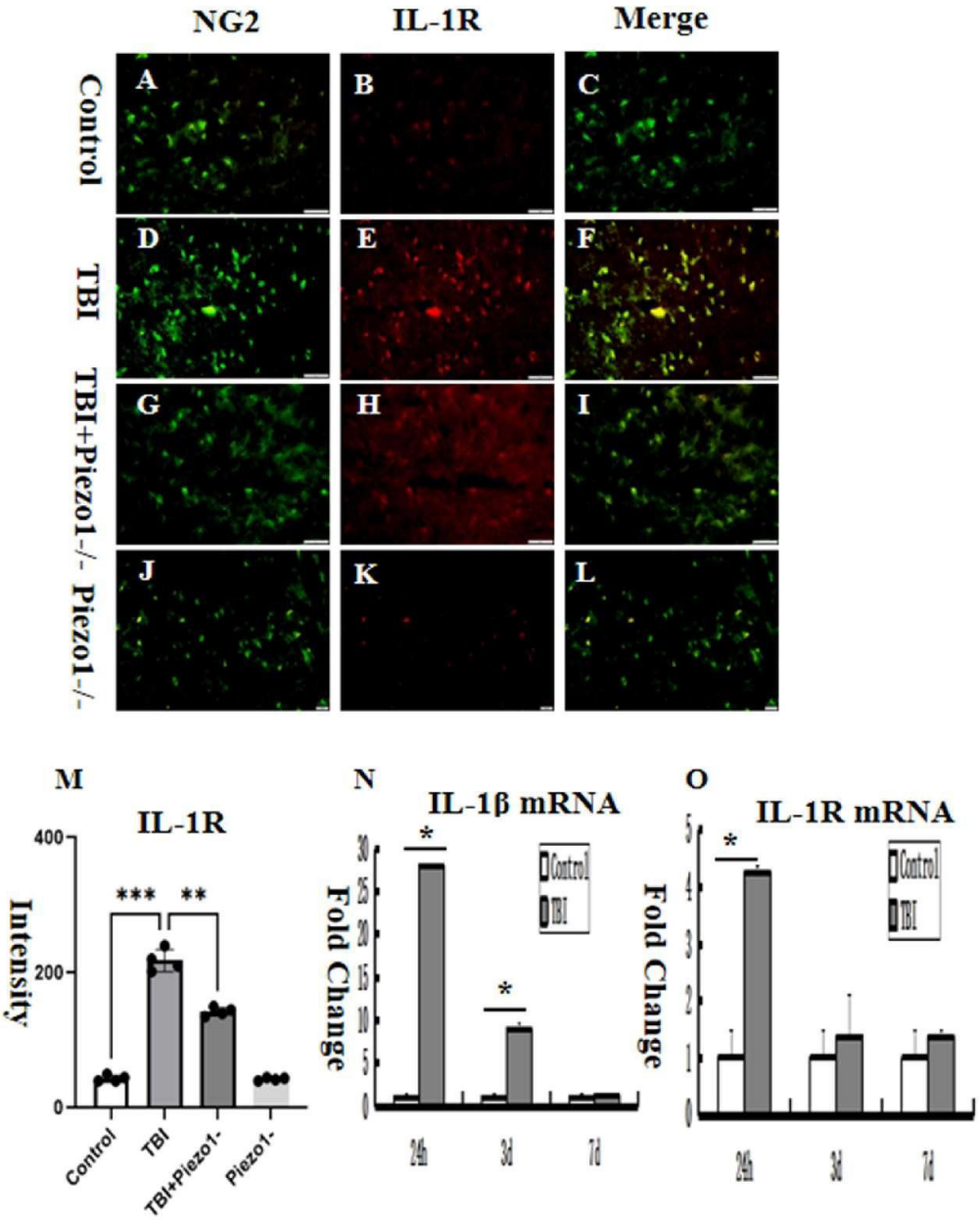


FIGURE 5. Piezo1 knockout alleviates expression of IL-1R on oligodendrocyte following TBI. In the hippocampal region of the brain tissue, IL-1R protein expression levels were up-regulated at 3 days after TBI injection when compared to the control group (A-M). However, administration of Piezo1 KO inhibited the expression of IL-1R. The results obtained from RT-PCR demonstrated that, in comparison to the control group, the TBI group exhibited a significant increase in the expression levels of IL-1β and IL-1R protein. Nevertheless, treatment with Piezo1 KO restored the expression of IL-1β and IL-1R (N and O).*P <0.05,**P <0.01, Scale bars: A–X 50 μm

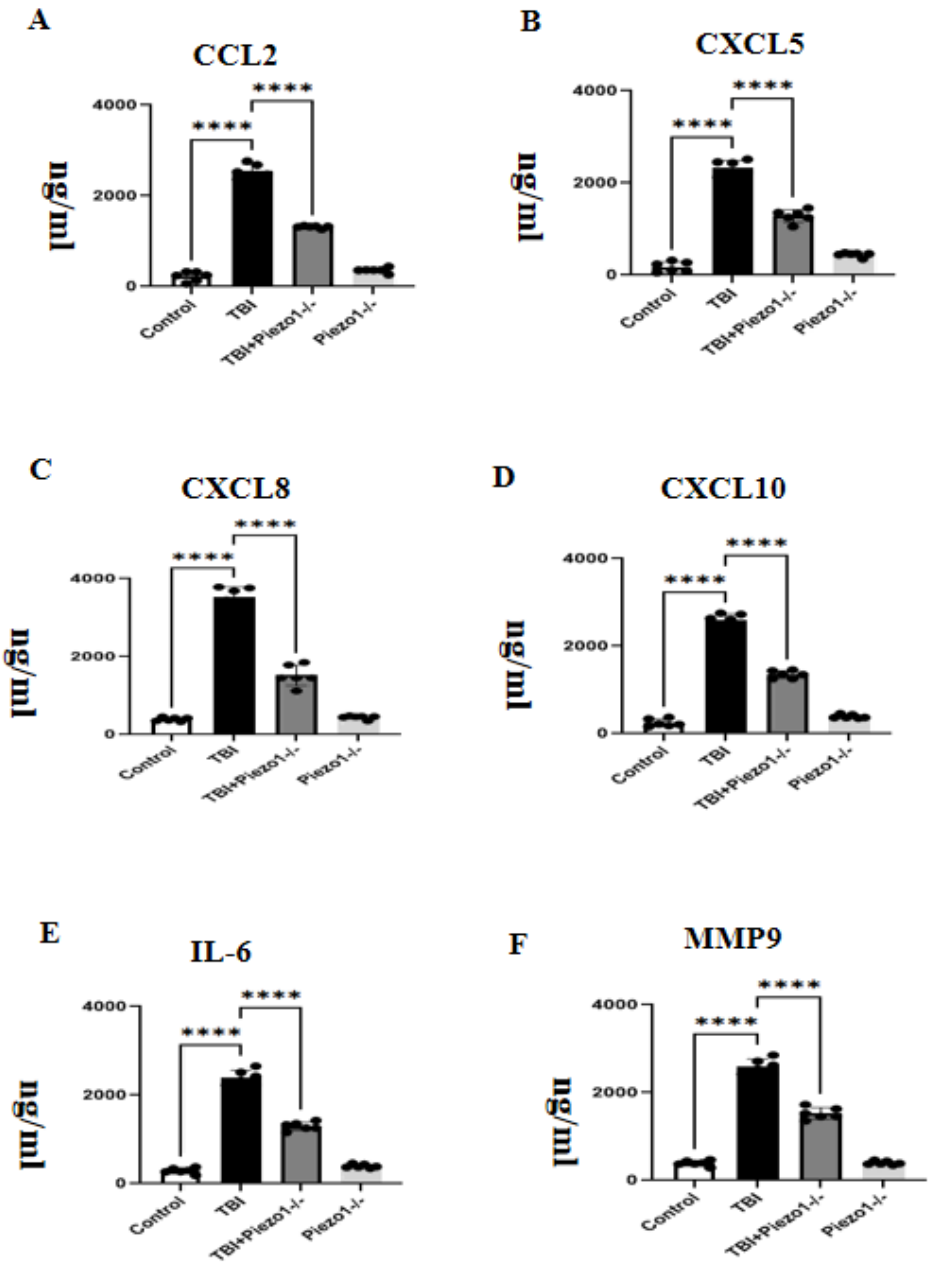


FIGURE 6. Piezo1 knockout inhibits M1 activation following TBI. ELISA was used to measure the expression of inflammatory factors secreted by M1-type microglial cells. The TBI group exhibited increased expression of CXCL2, CXCL3, CXCL5, IL-5, IL-6, and MMP9 compared to the control group. However, intervention with Piezo1 KO resulted in decreased expression of CCL2, CXCL5, CXCL5, CXCL10, IL-6, and MMP9. It is worth noting that administration of Piezo1 KO alone had no effect on the expression of inflammatory factors (A-F).****P <0.01

CCL2, CXCL5, IL-5, IL-6, and MMP9 decreased. However, piezo1 ko alone had no effect on the expression of inflammatory factors (Figure 6(A)-6(F)).

DISCUSSION

Traumatic brain injury (TBI) is a complex, heterogeneous condition that leads to powerful forces capable of causing extensive damage to the human brain (Hiwase et al. 2024; King et al. 2024). TBI can affect many aspects of an individual such as physical, cognitive, emotional, and behavioral. One of the most common and disabling sequelae is cognitive dysfunction, which includes impairments in memory, attention, executive function or information processing. Mechanism of TBI-induced cognitive dysfunction. The primary hit can cause direct damage to brain tissue, disrupting neural communication pathways and resulting in a series of pathological events that aggravate both acute brain injury and subsequent neurological dysfunctions. Secondary insults-such as pro-inflammatory cytokine release, activated microglia and disrupted blood-brain barrier integrity also underlie the cognitive deficits seen in TBI (Czerpaniak et al. 2024; Malarkey et al. 2024). Such secondary processes may survive for an extended time leading to chronic neuroinflammation and long-lasting cognitive impairment. After TBI, activated microglia play a dual role in which they remove cell debris as well as release inflammatory mediators that include cytokines and chemokines exacerbating neuroinflammation (Chen et al. 2024a; Li et al. 2024). Moreover, they are important for tissue repair and synaptic remodeling through their phagocytic activity as well as secretion of neurotrophic factors. In the context of traumatic brain injury (TBI), the CCL2, CCL7, CCL12, and CCR2 pathways are significantly and persistently upregulated in mice after injury. CCL2 regulates the complement system in microglial cells. TBI is a leading cause of global mortality and disability. In addition to direct cellular damage, delayed central nervous system damage following TBI presents typical symptoms including neuronal and glial cell apoptosis, axonal degeneration, mitochondrial damage, and subsequent disruption of neuroimmune interactions. A series of dynamic events involve the activation of glial cells residing in the central nervous system. The mouse TBI model has identified strong activation of microglial cells and emphasized sustained neurotoxicity mediated by microglia for up to a year after injury.

The response of microglia to TBI is multifaceted and influenced by factors including injury severity, age and comorbidities. Although microglia are polarized toward the pro-inflammatory (M1) phenotype during acute phase, secreting of IL-1β et al. may contribute to secondary brain damage in adjacent normal tissue. In later TBI stages, microglia transform to M2 anti-immune cells where they produce cytokines promoting high levels of the macrophage number by wildfire inhibiting pro-inflammatory response. However, by its very nature also this shift must be opposed to the overwhelming inflammation and instead support tissue repair and regeneration; here too proper timing of onset as well as severity is crucial. The discussion includes the increase in IL-6 levels after TBI, which is related to inflammation response and disruption of the blood-brain barrier (BBB). IL-6 can influence the immune response after TBI through various mechanisms: it can activate microglial cells and peripheral immune cells, increase the production of inflammatory mediators such as C-reactive protein and serum amyloid A, which may worsen inflammation and damage in the brain injury area. The elevation of IL-6 is associated with the disruption of the blood-brain barrier, which may lead to more immune cells and inflammatory mediators entering brain tissue, further exacerbating neuroinflammation. Several approaches to modulate microglial response and promote neuroregeneration in TBI are under investigation including pharmacological treatment, stem cell therapies as well as immunomodulatory agents. The expressions of CXCL10, CCL2, IL-6, and MMP9 inflammatory factors secreted by M1-type microglial cells were measured through immunofluorescence detection and ELISA. By contrast, the expression of CXCL10, CCL2, and Il-6 was significantly elevated in the TBI groups as comparing with that in control group. It has been added that Piezo1 is a mechanosensitive cation channel that acts in various cell types, including sensing mechanical forces and converting them into electrical or chemical signals. In the central nervous system, the function of Piezo1 is particularly important, especially in astrocytes and microglial cells. In oligodendrocytes, inhibiting Piezo1 can alleviate demyelination after brain hemorrhage. Following brain hemorrhage, the expression of Piezo1 increases in oligodendrocytes. Inhibiting Piezo1 can reduce oligodendrocyte apoptosis, protect myelin, and improve nerve function.

Defaunation with piezo1 ko intervention attenuated CXCL2, CXCL3, and IL-6 gene expression levels while the overproduction of neutrophils were correlated to increased mRNA abundance for Cxcl5 Il5,Mmp9protocols among other genes involved in immunity/defense response. However, the treatment of piezo1 ko alone had no effect on these inflammatory factor expression. Increasing the discussion on modulating the gut microbiota, such as using probiotics or fecal microbiota transplantation (FMT), may help improve intestinal barrier function after TBI, reduce inflammation, and thereby have a positive impact on recovery post-TBI. These therapeutic approaches may exert positive effects on neurological function recovery

post-TBI by enhancing the composition of gut microbiota, strengthening intestinal barrier function, and modulating signaling between the gut and the brain.(Ouyang et al. 2024; Sok et al. 2024).

LIMITATIONS

When investigating the causal relationship between inflammatory factors and traumatic brain injury (TBI) as well as cognitive impairment, bioinformatics analysis and Mendelian randomization are two important research methods. However, both of these methods have their own limitations. Bioinformatics analysis may struggle with handling complex biological systems and interactions among multiple factors, sometimes making it challenging to accurately deduce causality from large datasets. For example, while bioinformatics analysis can identify gene expression changes related to inflammatory factors, further experimental validation may be necessary to establish the direct causal relationship between these changes and TBI and cognitive impairment.

CONCLUSION

These findings indicate that Piezo1 could potentially serve as a therapeutic target in TBI to alleviate neuroinflammation.

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