Interferon-Stimulated Gene 15 Upregulation Precedes the Development of Blood–Brain Barrier Disruption and Cerebral Edema after Traumatic Brain Injury in Young Mice

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Abstract

Recent studies show that myosin light chain kinase (MLCK) plays a pivotal role in development of cerebral edema, a known complication following traumatic brain injury (TBI) in children and a contributing factor to worsened neurologic recovery. Interferon-stimulated gene 15 (ISG15) is upregulated after cerebral ischemia and is neuroprotective. The significant role of ISG15 after TBI has not been studied. Postnatal Day (PND) 21 and PND24 mice were subjected to lateral closed-skull injury with impact depth of 2.0 or 2.25 mm. Behavior was examined at 7 d using two-object novel recognition and Wire Hang tests. Mice were sacrificed at 6 h, 12 h, 24 h, 48 h, 72 h, and 7 d. ISG15 and MLCK were analyzed by Western blot and immunohistochemistry, blood–brain barrier (BBB) disruption with Evans Blue (EB), and cerebral edema with wet/dry weights. EB extravasation and edema peaked at 72 h in both ages. PND21 mice had more severe neurological deficits, compared with PND24 mice. PND24 mice showed peak ISG15 expression at 6 h, and PND21 mice at 72 h. MLCK peaked in both age groups at 12 h and co-localized with ISG15 on immunohistochemistry and co-immunoprecipitation. These studies provide evidence, ISG15 is elevated following TBI in mice, preceding MLCK elevation, development of BBB disruption, and cerebral edema.

Key words: behavior; cerebral edema; ISG15; MLCK; traumatic brain injury; young mice

Introduction

TRAUMATIC BRAIN INJURY (TBI) constitutes a major health and socioeconomic problem throughout the world.¹ In recent years, it has been estimated that 1.1 million people are treated in emergency departments. Of those, 5000 adult Americans are hospitalized for non-fatal TBI, and 50,000 adult Americans die from TBI.² An estimated 43.3% of Americans have residual disabilities one year after hospitalization with TBI. The most recent estimate of the prevalence of U.S. residents living with disability following hospitalization with TBI is 3.2 million.² Also, between 219,000 and 345,000 children experience TBI annually, and one in 30 newborns (0–30 days old) will experience a TBI by the time they reach age 16.³ Among children ages 0 to 19, each year an average of 62,000 patients sustain brain injuries requiring hospitalization as a result of motor vehicle crashes, falls, sports injuries, physical abuse, and other causes.⁴ Among children ages 0 to 14 years, TBI results in an estimated 435,000 emergency department visits, 37,000 hospitalizations, and 2685 deaths.⁴ Traumatic brain injury results in long-term delay in development, as well as cognitive and behavioral

impairment in children that lead to decreased productivity in adulthood.⁵

Cerebral edema is a well-known determinant of neurologic outcome following TBI in all ages but it is more severe in the young.^{5,6} Children who develop diffuse cerebral edema (53%) have a mortality rate up to 90% and usually die within 6 d, compared with children who do not develop cerebral edema.⁵ Diffuse cerebral edema develops more commonly in children than adults following TBI; however adults are more likely than children to develop a secondary edema resulting in a poor outcome.⁷ Adults who develop cerebral edema often have a more severe injury and poly-trauma, compared with children with the same amount of edema.⁷

The cytoskeleton is one of the structures that has been implicated in the disruption of the blood–brain barrier (BBB) and the development of cerebral edema.⁸ Myosin light chain kinase (MLCK) has been shown to affect the breakdown of the BBB and the development of cerebral edema, leading to a decline in neurological function.⁹ Whether TBI directly activates MLCK or whether other pathways are indirectly linked to MLCK is unknown. Several pathways have been shown to be involved in TBI and indirectly linked to MLCK.^{10,11} The

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ubiquitin (UB) superfamilies are well known to be involved in the disruption of multiple pathways related to TBI,¹² including immunology,¹³ metabolic,^{14,15} cardiovascular,¹⁶ neurologic,¹⁷ signal transduction,¹⁸ cytoskeletal organization,¹⁹ and deoxyribonucleic acid (DNA) replication and repair.²⁰ In addition, UB may be a potential early marker of outcomes following TBI in children.²¹

Interferon-stimulated gene 15 (ISG15) has been categorized as the first member of the UB-like superfamily of proteins.²² It was first discovered to be induced by type 1 interferons.²³ Due to its ability to cross-react with UB-specific antibodies, it was then referred to as ubiquitin cross-reactive protein²² until crystallography demonstrated that ISG15 is composed of two UB homology domains, each containing the signature B grasp fold found in UB and other UB-like proteins.²⁴ These two homology domains are joined by a linker peptide, thus categorizing ISG15 as a member of the UB superfamilies.²⁴ ISGylation has been shown to be elevated following TBI in mice,²⁵ and ISG15 also has been shown to be neuroprotective in a mouse model of stroke,²⁶ furthering the question of ISG15's role in TBI. Here, we investigate the role of ISG15 in the disruption of the BBB, as well as its relationship to MLCK and neurological sequelae following TBI in young mice.

Methods

Animals

All experiments were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee at the Louisiana State University Health Sciences Center, New Orleans, approved all experimental procedures. C57BL6 male mice at postnatal day (PND) 21 and PND24, and weighing 7–10 g and 10–13 g, respectively, were used for these experiments. All mice were randomly assigned to an impact depth or sham group.

Model of closed-head injury

Mice were anesthetized with 1% avertin (2, 2, 2 tribromethanol and tertiary amyl alcohol Sigma Aldrich); intraperitoneally (IP), 20 μ L/g, then non-invasively mechanically ventilated (Hugo Sachs Elektronik,) using an oral/nasal mask. Core temperature was monitored during surgery using a rectal probe (IT-4 Physitemp,) and maintained at 36.8–37.2°C by surface heating and cooling after impact using a heating pad under the home cage until mice were able to maintain their own body temperature. Mice were subjected to closed-skull traumatic brain injury using a stereotactically-guided electromagnetic compression device, as we described previously.⁹ In brief, a midline sagittal scalp incision was made and the periosteum reflected to reveal the appropriate landmarks. Immediately caudal to the bregma at 0 mm, a right lateral closed-skull impact was delivered via an electromagnetic impactor (Leica Microsystems- My Neuro Lab,) using a 3.0-mm steel tip impounder with a controlled velocity (3.0 ± 0.2 m/sec), impact depth (2.0 or 2.25 mm) and dwell time (0.01 sec). Sham mice received the same anesthesia, midline sagittal scalp incision, and steel tip impounder touching the skull, but with no impact.

Evaluation of blood–brain barrier integrity

The integrity of the BBB was investigated using Evans Blue (EB) extravasation, as we described previously.⁹ EB dye (2%, 10 μ L/g, IP Alpha Aesar; Fisher Scientific,) was injected to PND21 and PND24 (impact depth 2.0 and 2.25 mm) and sham mice ($n=6$ in each group) at 4 h prior to sacrifice at 6 h, 12 h, 24 h, 48 h, 72 h, and 7 d following impact. Mice were then anesthetized and perfused with saline. Whole brains were placed in tubes and immediately submerged in liquid nitrogen, then stored in a -80°C freezer until processing.

FIG. 1. Coronal brain section of mouse brain illustrating area of the impact (black dot) and region for Western blot and Evans Blue analysis.

Brain samples taken from below the impact site of 0 to -3.46 mm bregma (Fig. 1) and just to the right lateral of the sagittal suture were excised, weighed, sonicated in formamide (Sigma Aldrich) and then incubated overnight at 37°C . Samples were centrifuged and the supernatant aliquoted to a 96-well plate. Fluorescence was measured using an excitation of 600 nm and emission 680 nm with Spectramax M5 (Molecular Devices,). Fluorescence was analyzed by using Softmax Pro 5.4 (Analytical Technologies,).

Measurement of water content

PND21, PND24 (impact depths 2.0 and 2.25 mm), and sham mice ($n=6$ in each group) were sacrificed at 24 h, 48 h, 72 h, and 7 d, and wet/dry weights were measured at each time-point.⁹ Mice were sacrificed with CO_2 and whole brains were removed, immediately weighed, and then placed at 60°C for 3 d. Dried whole brains were then re-weighed and the ratio of wet/dry was calculated, as we described previously.⁹

Wire Hang test

PND21 and PND24 (impact depths 2.0 and 2.25 mm) and sham mice ($n=6$ in each group) were tested for motor strength on Day 7, as we described previously.⁹ Hang times (latency to fall in seconds [s]) for each treatment group were averaged. In brief, mice were individually placed on a wire cage lid until footing was established. The cage lid was then inverted and the time to fall was recorded during a 2-min maximum testing time. Animals that were able to climb topside were awarded the maximum 2-min score. The test was terminated at two minutes for those mice that neither climbed topside nor fell.

2-Object Novel Recognition test

This test is used to evaluate recognition memory on Day 7. PND21 and PND24 mice (impact depths 2.0 and 2.25 mm) and sham mice ($n=6$ in each group) were habituated to the testing room in their home cage for 24 h on Day 6 post-TBI. Mice were individually habituated to the testing black box for 10 min each. On Day 7 post-TBI, mice were placed individually in the black box with two identical objects for 10 min each. Four hours later, mice were placed individually in the black box with one familiar object and one new object. Each mouse was videotaped for 10 min, and the number of touches per object was recorded. Scoring was as follows: nose touch=1 point; two paws on object=2 points; and all four paws on top of object=3 points, as we previously described.⁹

Points were tallied and touches to old versus new objects were compared. An observer blinded to the impact groups conducted video analysis.

Western blots

Brain tissue samples (100 mg) from under the impact site (Fig. 1) or comparable location of sham from time-points 6 h, 12 h, 24 h, 48 h, 72 h, and 7 d, animals were homogenized in 1 × RIPA lysis buffer (Millipore,) with protease inhibitor (Sigma Aldrich). Protein concentrations were determined with Bicinchoninic Acid Protein Assay Kit (Pierce/Thermo Scientific.). ISG15 protein lysates (30 µg) were fractioned by 4%-12% Bis-Tris gels (Novex/Life Technologies,) and MLCK protein lysates (90 µg) were fractioned by 3–8% Tris-Acetate gels (Novex/Life Technologies), with both then transferred using the iBlot system, (Life Technologies.). ISG15 blots were probed with ISG15 rabbit serum (Washington University St. Louis, courtesy of Dr. D. Lenschow) and goat anti-rabbit immunoglobulin G (IgG) secondary (Life Technologies). MLCK blots were probed with rabbit monoclonal primary antibody (Abcam,) and goat anti-rabbit IgG secondary (Life Technologies). Both were developed using Supersignal West Femto Maximum Sensitivity Substrate (Thermo Scientific) and imaged with GE ImageQuant LAS 4000 (GE Healthcare Life Sciences.).

Immunohistochemistry

Immunohistochemistry was performed on PND21 mice at 24 h, as we reported previously.⁹ In brief, the animals were sacrificed with 1% Avertin (100 µL/g), perfused with normal saline by perfusion pump 10 mL/min for 2 min. Whole brains were removed, immersed in 4% paraformaldehyde, and then cryopreserved. Serial sections 20-micron thick extending from bregma 0 mm to –3.46 mm were prepared. The sequence for antibody processing was the same for each brain. All sections were rinsed, blocked with 10% normal swine serum, and incubated overnight at room temperature with primary antibodies: ISG15 rabbit serum (Lenschow Lab.) 1:400 and MLCK mouse monoclonal IgG (Sigma Aldrich) 1:150. Negative control sections were incubated in normal serum and phosphate-buffered saline in place of primary antibody. ISG15 was detected using Alexa flour 488 (Life Technologies) 1:200. MLCK was detected using Dylight 594 (Thermo Scientific) 1:200 and 4',6-diamidino-2-phenylindole (DAPI; Life Technologies). Slides were photographed using a Deconvolution Axioplan Z imaging microscope with a Sensicam QE and Slidebook 5.0 at 20×.

Co-immunoprecipitation

Co-immunoprecipitation was performed to assess protein-protein interaction between ISG15 and MLCK. We incubated 500 µg of PND24 TBI lysate from 6 h, 24 h, and 7 d time-points for 1 h at 4°C with either MLCK rabbit polyclonal (Abgent,) or ISG15 mouse monoclonal (Santa Cruz Biotechnology,) primary antibody. A total of 20 µL of Protein A/G PLUS-Agarose Immunoprecipitate Reagent (Santa Cruz Biotechnology) was added and incubated overnight at 4°C. ISG15 IP and MLCK IP were separated on a 3–8% Tris-Acetate mini-gel and transferred as described above. The ISG15 IP blot was probed with MLCK rabbit monoclonal primary antibody (Abcam) and anti-rabbit IgG (Life Technologies). The MLCK IP blot was probed with ISG15 mouse monoclonal primary antibody (Santa Cruz Biotechnology) and goat anti-mouse IgG (Millipore). Blots were developed as described above.

Statistical analysis

Values are expressed as mean ± standard error of the mean for each group. Tests for normality were performed for each data set. Parametric tests were used when the data were normal, and non-parametric tests were used if the data were not normal. One-way

analysis of variance (ANOVA) or the Kruskal-Wallis test for nonparametric analysis was performed to compare three or more groups. Bonferroni's multiple comparison procedure (or Dunn's procedure for nonparametric analysis) was used for post hoc analysis. For the analysis of the 2-Object Novel Recognition we used specifically Kruskal Wallis with multiple comparisons analyzing all groups per age at one time comparing both old to new and new to old per impact depth. Significance was defined as $p < 0.05$ for all tests. Prism 5.0 Software (GraphPad Software, Inc.,) was used for statistical analyses.

Results

Evans Blue extravasation

Representative brain sections from PND21 mice (Fig. 2A) and PND24 mice (Fig. 2B) at each impact depth showed more diffuse extravasation of EB in PND21 mice, compared with the PND24 mice. PND21 mice (Fig. 2C) showed extravasation of EB through 7 d, compared with PND24 mice (Fig. 2D), which returned to baseline by 7 d. Both ages showed peak extravasation at 72 h (Fig. 2).

Wet/dry weight

PND21 and PND24 mice showed increased wet/dry weight ratios with PND21 beginning earlier and resolving later than PND24 (Fig. 3). Both ages showed peak ratios at 72 h (Fig. 3).

Behavior tests

2-Object Novel Recognition. PND21 TBI mice (Fig. 4A) at both depths showed a preference for the old object, compared with the new object. PND21 sham mice had a greater preference for the new object over the old object. PND24 TBI mice (Fig. 4B) at 2.25 mm preferred the old object to the new object but no preference between the old and new objects was observed in the PND24 2 mm mice. PND24 sham mice showed a greater preference for the new object over the old object.

Wire Hang test. PND21 mice (Fig. 3C) and PND24 (Fig. 3D) mice showed overall similar patterns regarding latency to fall. The TBI 2.25mm mice exhibited the shortest latency to fall times, followed by the TBI 2 mm mice, with sham mice exhibiting the longest latency to fall times (Fig. 4).

Western blot: ISG15 analysis

Figure 5A and Figure 5B (PND21 and PND24 mice, respectively) show representative blots from 6 h, 72 h, and 7 d after TBI. ISG15-conjugated proteins (proteins reactive with ISG15 antibodies) increased progressively from 6 h to 7 d in PND21 mice, and ISG15-conjugated proteins decreased progressively from 6 h to 7 d in PND24 mice. PND21 mice (Fig. 5C) showed increasing expression of free ISG15 beginning at 6 h and peaking at 72 h, resulting in significance at both depths and between depths. Expression levels of free ISG15 reached baseline at 24 h and 7 d. PND24 mice (Fig. 5D) showed decreasing expression of free ISG15 beginning with a peak at 6 h and declining through 72 h, resulting in significance at both depths and between depths. Expression levels of free ISG15 reached baseline at 48 h and 7 d.

Immunohistochemistry and Western blot: MLCK and ISG15 analysis

Figure 6 shows representative images from PND21 (2.25 mm depth) at 24 h after TBI. ISG15 (Fig. 6A), MLCK (Fig. 6B) and

co-localization of ISG15 with MLCK (Fig. 6C) were analyzed using immunohistochemistry and western blot. PND21 mice (Fig. 6D) and PND24 mice (Fig. 6E) showed increasing expression of MLCK beginning at 6 h and peaking at 12 h, then returning to baseline by 48 h. Both depths showed significance, compared with the sham, but they showed no significance when compared with each other.

Co-immunoprecipitation

A MLCK immunoprecipitation blot (Fig. 6F) probed with ISG15 showed bands at 210 and 108, corresponding to the MLCK proteins in the PND24 mice (2.25 mm) at 6 h, 24 h, and 7 d after TBI. ISG15 immunoprecipitation blot (Fig. 6G) probed with MLCK showed bands at 210 and 108, also correlating to the bands of MLCK.

FIG. 4. Object Novel Recognition: Postnatal Day 21 (PND21) TBI mice (**A**) showed a preference for the old object, compared with the new object, at both depths. Sham mice had a greater preference for the new object over the old object. PND24 mice (**B**) showed preference for the old vs. new object in the 2.0 mm and no preference between old and new object in the 2.25 mm traumatic brain injury (TBI) mice. The sham mice show a greater preference for the new object over the old object. Wire Hang: PND21 mice (**C**) and PND24 mice (**D**) both show decreasing latency to fall, with sham mice having the longest hang time. Hang time decreased for mice with 2.0 mm-depth TBI, and mice with 2.25 mm-depth TBI fell almost immediately; there was a notable significance between depths for both PND21 and PND24. * Significance with new object preferred to old object, &- Significance with old preferred to new object. * or & $p < 0.01$ ** or && $p < 0.001$ *** or &&& $p < 0.0001$. One-way analysis of variance, non-parametric with multiple comparisons. Mean $\pm$ standard error of the mean.

FIG. 5. ISGylation. Representative blots from Postnatal Day 21 (PND21) mice (**A**) and PND24 mice (**B**) at 6 h, 72 h, and 7 d after TBI. ISG15-conjugated proteins increased progressively from 6 h to 7 d in PND21 mice and ISG15-conjugated proteins decreased progressively from 6 h to 7 d in PND24 mice. Free ISG15 in PND 21 mice (**C**) show an increasing expression of free ISG15 beginning at 6 h with peaks at 12 h and at 72 h, with significance at both depths and between depths. Free ISG15 reaches baseline at 24 h and 7 d. Free ISG15 in PND 24 mice (**D**) show a decreasing expression of free ISG15 over time beginning at 6 h with peaks at 6 h and 72 h, with significance at both depths and between depths. Free ISG15 reaches baseline at 48 h and 7 d. * - 2.25 mm or 2.0 mm impact depth, compared with sham, # - 2.25 mm, compared with 2.0 mm. * or # $p < 0.01$ ** or ## $p < 0.001$ *** or ### $p < 0.0001$. One-way analysis of variance, non-parametric with multiple comparisons. Mean $\pm$ standard error of the mean.

FIG. 6. Protein–protein interaction for myosin light chain kinase (MLCK) and interferon-stimulated gene 15 (ISG15). Representative images from postnatal day 21 (PND21) mice (2.25 mm) at 6 h, 24 h, and 72 h after traumatic brain injury. (A) ISG15 fluorescent green. (B) MLCK fluorescent red. (C) Orange co-localization of ISG15 and MLCK with 4',6-diamidino-2-phenylindole blue. Scale bar, 25 μ m. Arrows showing co-localization. MLCK in both PND21 (D) and PND24 mice (E) show an increasing expression of MLCK beginning at 6 h and peaking at 12 h, then returning to baseline by 48 h. Both depths, compared with sham, show significance, but no significance occurs between depths. * - 2.25 mm or 2.0 mm impact depth, compared with sham, -. * $p < 0.01$ ** $p < 0.001$. One-way analysis of variance, non-parametric with multiple comparisons. There is a similar pattern of expression of MLCK in both PND21 and PND24 mice. Mean $\pm$ standard error of the mean. Co-Immunoprecipitation: Immunoprecipitation (F) with MLCK and blot probed with ISG15 (G). Both PND21 and PND24 show MLCK bands at 210 and 108. Color image is available online at www.liebertpub.com/neu

Discussion

It is well known that TBI results in the following: stimulation of the immune system (as seen by release of inflammatory mediators)²⁷; stimulation of the endocrine system (as evidenced by development of syndrome of inappropriate antidiuretic hormone post-traumatic diabetes insipidus);²⁸ altered metabolism (as seen with elevated free fatty acids in the CSF);¹⁵ and altered oxygen metabolism.²⁹ Recently, we showed that the cytoskeleton plays a pivotal role in the development of cerebral edema and the decline in neurological function following TBI in PND24 mice.⁹ Here, we compared the effect of age, severity of injury, and the role of the cytoskeleton on neurological outcomes to further understand the signaling pathway leading to long-term neurological sequelae.

PND21 mice showed a more diffuse disruption of the BBB, where each area of disruption was larger than the comparable impact depth in the PND24 mice and where the 2.25 mm depth was more broadly disruptive. In contrast, PND24 mice showed a focal disruption of the BBB around the area of impact, where the area of

disruption was greater after the 2.25 mm depth injury than the 2 mm depth injury. This disruption in the BBB translates to the global cerebral edema seen in both PND21 and PND24 mice.

The edema peaked at 72 h in both PND21 and PND24 TBI mice, with more variability in the PND21 mice, as based on the error bars, and more edema in the PND21 2 mm mice at 48 h, compared with the PND24 mice at the same point in time. Also, the resolution of edema was slower in the PND21 mice, based on the significance at Day 7 for the 2.25 mm depth. These data indicate the younger mice are more vulnerable to TBI under same condition in part due to disruption of the BBB and the slower resolution of the cerebral edema.

The slower resolution of cerebral edema could be one of the reasons for the poorer performance in the neurological tests in the PND21 mice, compared with the PND24 mice. There was an increase in motor impairment in PND21 mice, compared with PND24 mice. However, given that the PND21 sham mice also were more apt to fall, it was more likely a reflection of developmental strength. Both PND21 and PND24 TBI mice had a more significant difference in motor impairment, compared with shams, with the 2.25 mm depth being greater than 2 mm. The PND21 mice showed a greater

difference in motor impairment between the 2 mm and the 2.25 mm, compared with the PND24 mice; again, this was likely due in part to the slower resolution of the cerebral edema. These results also translated to the cognitive testing, which showed that both PND21 and PND24 sham mice were able to recognize new objects more than old objects, and that both of these groups had a similar overall number of touches. However, both PND21 and PND24 mice at 2.25 mm preferred the old object, compared with the new object, but only the PND21 mice had the same significance at the 2 mm depth. The PND24 mice at 2 mm showed a similar investigation pattern for the new and old objects, and had less overall touches of either object at 2 mm than either the 2.25 mm or sham mice. The significance of fewer touches at the 2 mm level, compared with the 2.25 mm level in both ages is unclear, but this could be due to an as yet unknown difference in the signaling pathway resulting from the two different impacts. This supports the theory there are potential changes involving the BBB, making younger mice more vulnerable to TBI.

In our previous work, we showed that MLCK played a pivotal role in the development of cerebral edema and that inhibiting MLCK resulted in preservation of neurological outcome.⁹ ISG15 has been shown to post-translationally modify actin, converting it from F actin to stress actin in breast cancer.³⁰ Stress actin is involved in the breakdown of barriers through disruption of tight junctions.³¹ MLCK is known to phosphorylate myosin light chain, which then interacts with the actin head to convert F actin to stress actin, leading to disruption of barriers.³¹ In an effort to understand the stimulus of MLCK following TBI, we examined the relationship between MLCK and ISG15.

Both PND21 and PND24 mice showed a period of significant decrease in ISG15, only to have it rise again for mice that received a deeper impact (2.25 mm depth). This would suggest a potential cyclic nature of the ISG15 that requires further investigation. PND24 mice showed evidence of ISGylation upregulation at time-points in proximity to the injury time, compared with PND21 mice, which showed a delayed elevation of free ISG15 peaking at 72 h, as well as delayed ISGylation, suggesting a developmental component to the process of ISGylation. MLCK in both PND21 and PND24 mice peaked after up regulation of ISG15. This suggests that ISG15 may stimulate MLCK, but further studies that examine cytoskeletal interactions would need to be performed to clarify this relationship.

We showed the BBB has a more diffuse breakdown and that cerebral edema has a slower resolution in the PND21 mice, compared with the PND24 mice, following TBI. Thus in the motor and behavior tests, this translated to worse scores that were related to both age and the depth of the impact. We also showed that ISG15 is upregulated before MLCK, and that ISGylation is increased in PND24 mice before PND21 mice. In addition, MLCK and ISG15 showed co-localization with protein–protein interactions that fluctuate over time. The actual significance of the interaction between MLCK and ISG15 needs further examination.

There are many potential causes of worsening neurological decline in younger age children. The skull in younger ages is more compliant; therefore, distributing the force of the impact more broadly across the skull; this could be the explanation for the more diffuse distribution of the Evans Blue in the PND21 mice, compared with PND24 mice. Broader distribution of force can lead to greater tissue loss and cell death. Diffuse axonal injury, disruption of cell signaling and synaptic connectivity, as well as mitochondrial derangements, are well-known consequences of TBI all contributing to the neurological decline following TBI. Our previous work showed limiting cerebral edema preserved neurological outcome.

How the BBB disruption and cerebral edema exacerbate these injuries needs further examination. Uncovering the interactions of ISG15 with these many pathways will expand the understanding of signal transduction and eventually translate to improved neurological outcome following TBI in children.

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Author Disclosure Statement

No competing financial interests exist.

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