SUPPLEMENTARY MATERIALS

New Astroglial Injury Defined Biomarkers for Neurotrauma Assessment

Julia Halford[1], Sean Shen[2], Kyohei Itamura[1], Jaclynn Levine[1], Albert C. Chong[1], Gregg Czerwieniec[2], Thomas C. Glenn[4], David A. Hovda[4], Paul Vespa[5], Ross Bullock[6], Dalton W. Dietrich[7], Stefania Mondello[8], Joseph A. Loo[2, 3] and Ina-Beate Wanner[1]

[1]Semel Institute for Neuroscience and Human Behavior, [2]Department of Chemistry and Biochemistry, [3]Department of Biological Chemistry, [3]UCLA Molecular Biology Institute, and UCLA/DOE Institute for Genomics and Proteomics, [4]Department of Neurosurgery, Brain Injury Research Center, Department of Molecular and Medical Pharmacology, [5]Department of Neurology, UCLA-David Geffen School of Medicine, Los Angeles; [6]Department of Neurological Surgery, Jackson Memorial Hospital; Miami; [7]The Miami Project to Cure Paralysis, University of Miami-Miller School of Medicine; [8]Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy

Corresponding Author: Ina B. Wanner, PhD

Semel Institute for Neuroscience and Human Behavior

635 Charles E Young Drive South, NRB 260, UCLA, Los Angeles, CA 90095

E-mail: iwanner@mednet.ucla.edu

Phone: 310 825 8847

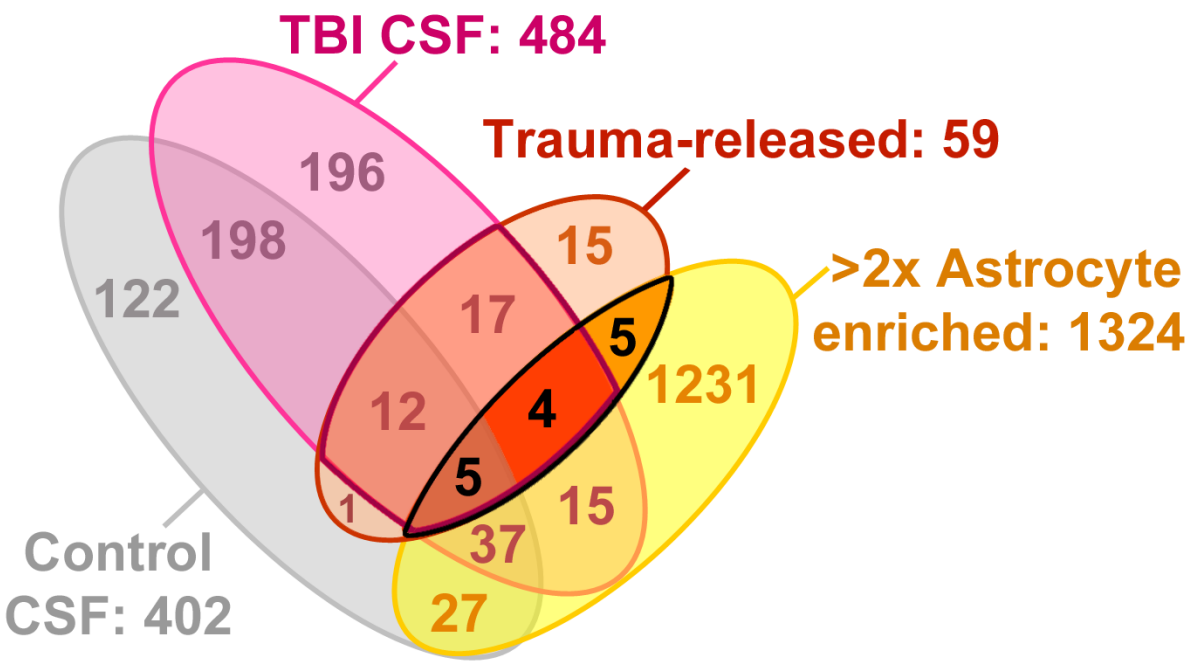
SUPPLEMENTARY FIGURES: 4

SUPPLEMENTARY TABLES: 2 (Proteomic table as separate file)

SUPPLEMENTARY REFERENCES

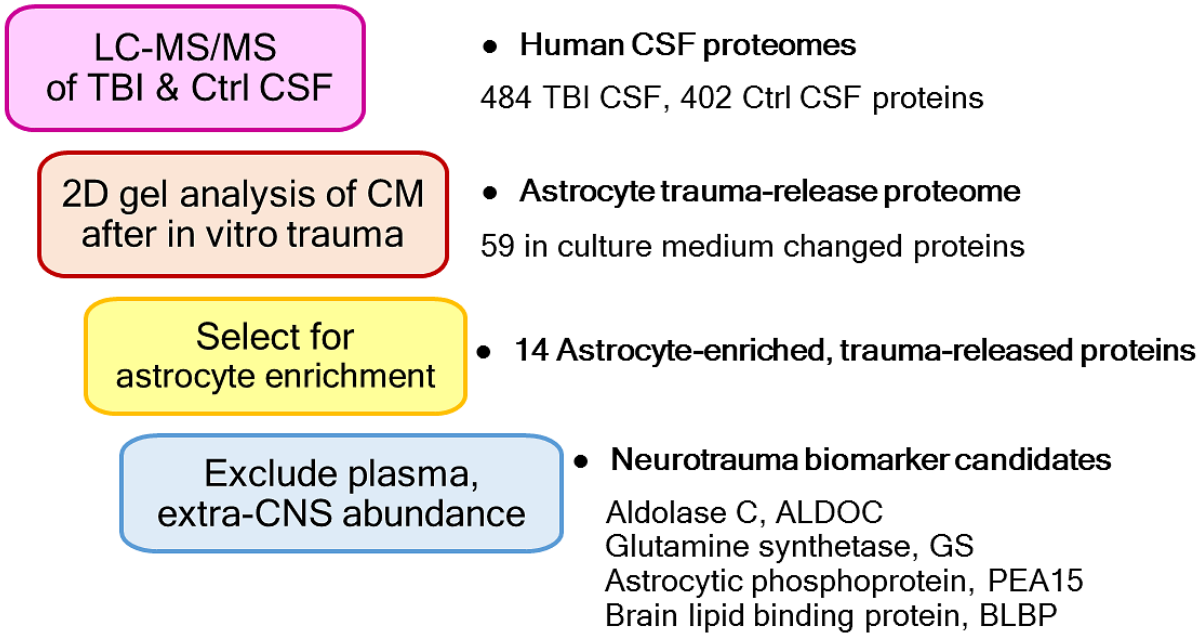
SUPPLEMENTARY FIGURES

S2: Venn diagram illustrates astrocyte proteomic signature of neurotrauma in human CSF.



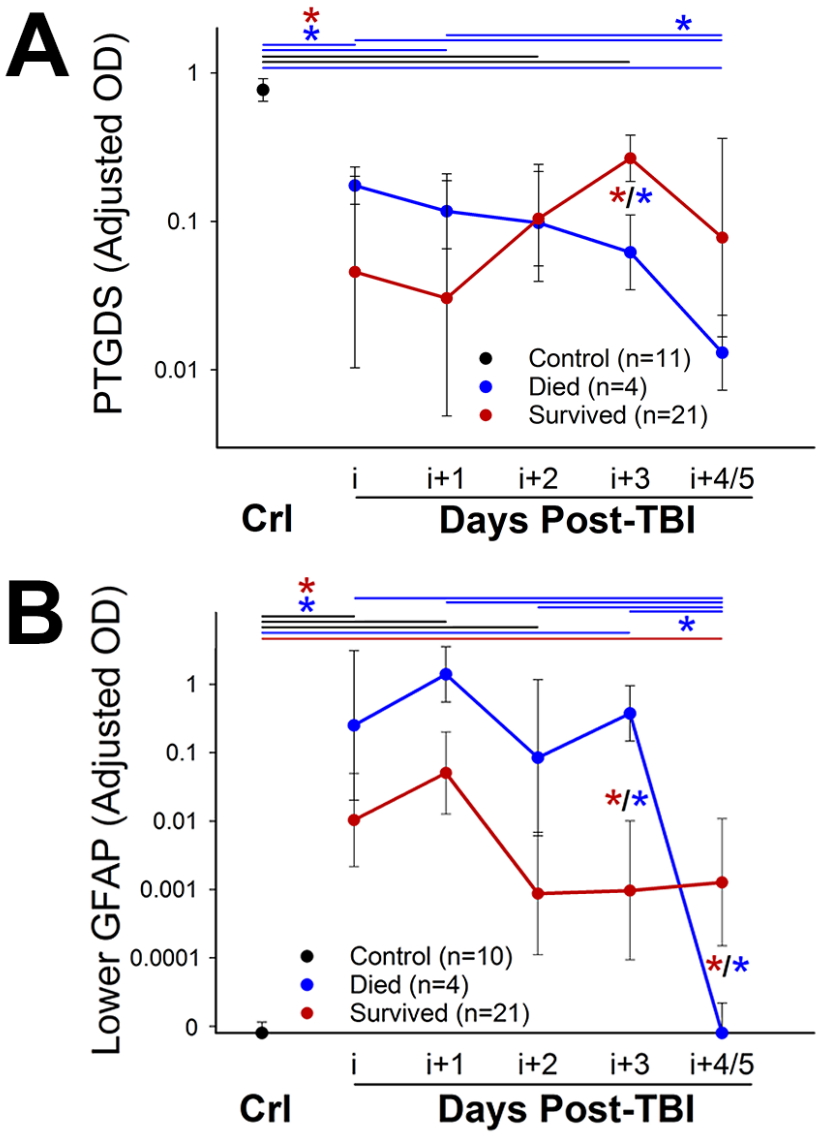
Venn diagram documents LC-MS/MS proteomes of CSF from 25 samples of 17 severe TBI patients (including injury day and subsequent four post-injury days, 484 proteins) and nine healthy subjects (402 proteins, Crl, Supplementary Table 1). A published *in vitro* astrocyte trauma-release proteome included 59 proteins that are significantly released into fluids 30min and 5h post-stretching[1]. Of this acute 'traumatome,' 38 proteins (64%, purple outline) overlapped with clinical CSF proteomes. A subset of 14 proteins in this overlap was at least 2-fold astrocyte-enriched[2] (black outline). GFAP was identified, but not quantified in the acute proteomic studies using two-dimensional gel electrophoresis; hence it is included among the 15 astrocyte-enriched and TBI CSF proteins. Aldolase C (ALDOC) was identified among five candidates present in TBI and control CSF (brown triangle). Among four proteins exclusive in TBI CSF was glutamine synthetase (GS, also known as GLNA, red box). Among the additional five trauma-released, astrocyte-enriched proteins (orange triangle) were astrocytic phosphoprotein 15 (PEA15) and brain lipid binding protein (BLBP, also called brain fatty acid binding protein, FABP7), which were considered despite their absence in CSF proteomes since shotgun LC-MS/MS provides limited sensitivity.

S3: Flow chart illustrates candidate selection strategy for neurotrauma biomarkers.



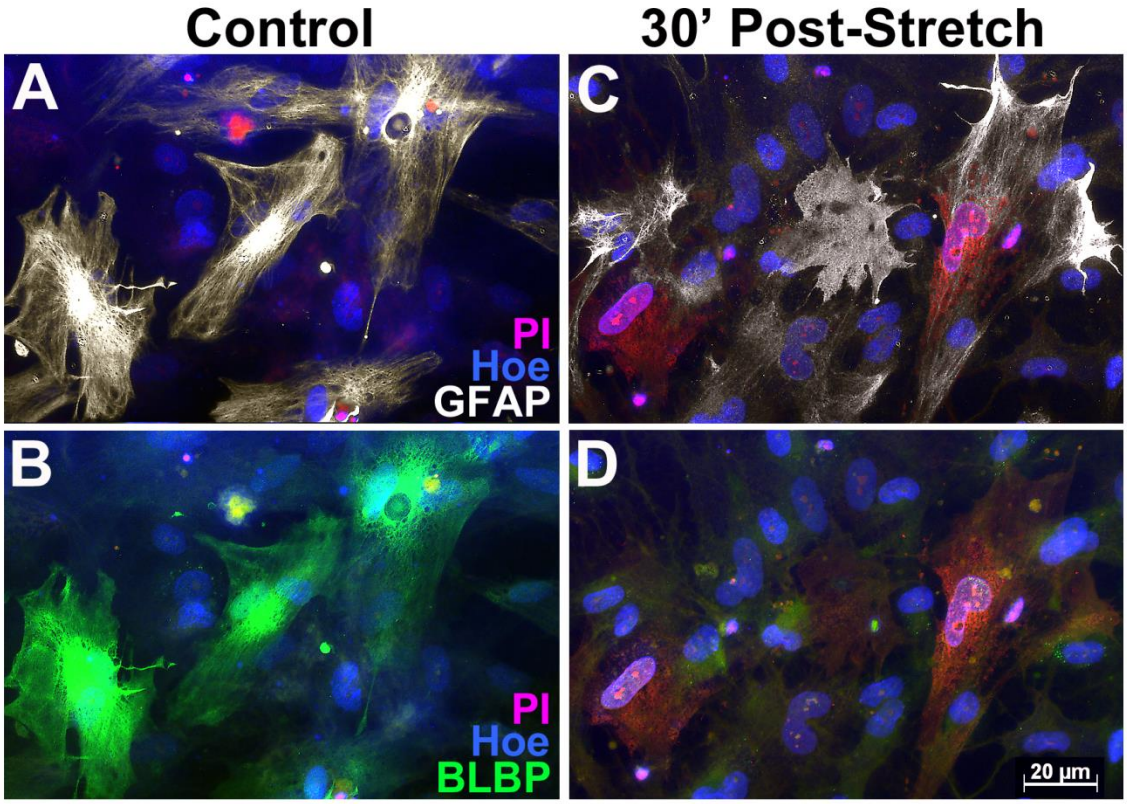
The flow chart shows steps used to arrive at new candidate astroglial neurotrauma biomarkers. First, TBI and control CSF proteomes generated by LC-MS/MS were compiled and compared (Supplementary Table 6). Next, overlap was determined between CSF proteomes and a previously identified list of 59 trauma-changed proteins acutely released from stretched astrocytes[1]. Astrocyte-enriched proteins (2-fold or greater) were then selected from the overlap between CSF and the trauma model proteome lists using published astrocyte gene expression arrays[2]. From the resulting 14 astrocyte-enriched and trauma-released proteins, those present in healthy donor plasma were removed, including coactosin-like protein 1, heat shock cognate 71kDa protein, vinculin, apolipoprotein E, clusterin and lactate dehydrogenase B[3, 4]. GFAP is present in healthy donor plasma, but was included as a known biomarker candidate for comparison[4]. Proteins with dominant expression outside the CNS were also excluded: transgelin, F-box only protein 2 and N, N-dimethyl arginine dimethyl aminohydrolase 1[5]. Resulting astroglial neurotrauma biomarker candidates were ALDOC, GS, BLBP and PEA15, all with predominant CNS expression.

S4: PTGDS and small GFAP-BDP CSF levels differ between TBI survivors and nonsurvivors.



Shown are geometric mean CSF levels of **(A)** PTGDS and **(B)** small GFAP-BDPs (18-25kD) in Controls (black), TBI survivors (red) and nonsurvivors (blue) with lower and upper bound error bars (95% confidence interval). **(A)** Mean PTGDS levels decreased early post-injury variably in survivors and more consistently in nonsurvivors (*, $p<0.01$); levels gradually recovered in survivors, resulting in significantly higher means on i+3 compared to nonsurvivors of TBI (*/*, $p=0.04$). **(B)** Mean CSF post-injury levels of small GFAP-BDPs were consistently higher in nonsurvivors versus survivors of TBI (i: 24-fold; i+1: 28-fold; i+2: 97-foldand i+3: 388-fold, */*$p=0.02$). By comparison, total GFAP adjusted densities did not result in significant differences between nonsurvivors and survivors of TBI in this small cohort (i: 2.6-fold; i+1: 2-fold; i+2: 22-fold; i+3: 11-fold, not plotted). Adjusted ODs: preliminary analyses were repeated measures ANOVA, mixed model adjusted for correlation over time, with non-constant intraclass variance (n=number of patients/subjects on x-axes[6].

S5: GFAP and BLBP are co-expressed in human astrocytes but respond differently to stretching.



A) Population of human neocortical control astrocytes shows robust GFAP **(**white) and **(B)** BLBP **(**green) expression. **C)** Wounded astrocytes 30min post-stretching had GFAP filament loss and accumulation in processes**. D)** In contrast, BLBP signals were depleted in wounded astrocytes 30min post-stretching**.** Wounded cells have PI-positive nuclei (red). Bar=20µm.

TABLE 7: Biomarker panel Spearman correlation from CSF of severe TBI patients.

Variable	by Variable	Spearman, r_s	p value	observations	
APOB	GFAP small BDPs	**0.898**	< 0.001	*42*	**Very strong**
S100β	GFAP small BDPs	**0.87**	< 0.001	*54*	
APOB	S100β	**0.847**	0	*44*	
PEA15	BLBP	**0.8054**	<.0001	*46*	
GFAP	GFAP small BDPs	**0.757**	< 0.001	*64*	**Strong**
S100β	GFAP	**0.7391**	<.0001	*54*	
APOB	GS	**0.726**	0	*44*	
BLBP	ALDOC	**0.6816**	<.0001	*56*	
PEA15	S100β	**0.6772**	<.0001	*43*	
GS	ALDOC	**0.6724**	<.0001	*53*	
APOB	BLBP	**0.638**	0	*44*	
GS	BLBP	**0.603**	<.0001	*49*	
APOB	ALDOC	**0.602**	0	*46*	
BLBP	GFAP small BDPs	**0.59**	< 0.001	*54*	**Moderate**
BLBP	S100β	**0.5833**	<.0001	*51*	
GS	S100β	**0.5826**	<.0001	*46*	
PEA15	GFAP	**0.5755**	<.0001	*47*	
GS	GFAP small BDPs	**0.573**	< 0.001	*51*	
PEA15	GFAP small BDPs	**0.572**	< 0.001	*47*	
PEA15	ALDOC	**0.5589**	<.0001	*49*	
GS	ALDOC 38kD BDP	**0.549**	0.0009	*33*	
APOB	GFAP	**0.541**	0.0002	*42*	
PEA15	GS	**0.5334**	<.0001	*49*	
BLBP	ALDOC 38kD BDP	**0.532**	0.0003	*41*	
BLBP	GFAP	**0.5149**	<.0001	*54*	
APOB	PEA15	**0.506**	0.0002	*48*	
ALDOC	ALDOC 38kD BPD	**0.506**	< 0.001	*59*	
ALDOC	GFAP small BDPs	**0.477**	< 0.001	*61*	
ALDOC	S100β	**0.4503**	0.0005	*56*	
ALDOC	GFAP	**0.3927**	0.0017	*61*	**Weak**
GS	GFAP	**0.3275**	0.0190	*51*	P<0.05
PEA15	ALDOC 38kD BDP	**0.309**	0.0749	*34*	**Very weak**
APOB	ALDOC 38kD BDP	**0.261**	0.1353	*34*	
PTGDS	ALDOC	**0.017**	0.893	*61*	
PTGDS	ALDOC 38kD BDP	**-0.024**	0.8779	*42*	
ALDOC 38kD BDP	GFAP small BDPs	**-0.029**	0.8628	*39*	
S100β	ALDOC 38kD BDP	**-0.04**	0.808	*39*	**None**
PTGDS	GS	**-0.13**	0.3641	*59*	
PTGDS	APOB	**-0.183**	0.2344	*44*	
PTGDS	BLBP	**-0.198**	0.1473	*57*	
GFAP	ALDOC 38kD BDP	**-0.201**	0.221	*39*	
PTGDS	GFAP small BDPs	**-0.251**	0.055	*59*	
PTGDS	PEA15	**-0.307**	0.0356	*47*	P<0.05
PTGDS	S100β	**-0.314**	0.0248	*54*	
PTGDS	GFAP	**-0.446**	0.0004	*58*	**Moderate**

Spearman rank correlation coefficients (r_s) are given for all pairs of new and known astroglial neurotrauma biomarkers, APOB and PTGDS with p-values and number of CSF samples analyzed. Coefficients 0.8 to 0.99 = very strong, 0.6 to 0.8 = strong, 0.4 to 0.6 = moderate, <0.4 = weak and <-0.3 = divergent. Small GFAP-BDPs (25-18 kD) and 38kD ALDOC-BDP signals varied from their main bands and were treated as additional biomarkers.

SUPPLEMENTARY REFERENCES

1. Levine J, Kwon E, Sondej M, et al. Traumatically injured astrocytes release a proteomic signature modulated by STAT3 dependent cell survival. *Glia*. 2016; 64: 668-94.
2. Cahoy JD, Emery B, Kaushal A, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2008; 28: 264-78.
3. Schenk S, Schoenhals GJ, de Souza G and Mann M. A high confidence, manually validated human blood plasma protein reference set. *BMC Med Genomics*. 2008; 1: 41.
4. Omenn GS, States DJ, Adamski M, et al. Overview of the HUPO Plasma Proteome Project: results from the pilot phase with 35 collaborating laboratories and multiple analytical groups, generating a core dataset of 3020 proteins and a publicly-available database. *Proteomics*. 2005; 5: 3226-45.
5. Kapushesky M, Adamusiak T, Burdett T, et al. Gene Expression Atlas update--a value-added database of microarray and sequencing-based functional genomics experiments. *Nucleic acids research*. 2012; 40: D1077-81.
6. Crowder MJ and Hand DJ. Analysis of repeated measures. *Monographs on statistics and applied probability*. 1st ed. London ; New York: Chapman and Hall, 1990, p. 1-59.