Research Goals: To examine putative protein markers of traumatic axonal injury (AI) in order to elucidate the neuropathology of mild traumatic brain injury (mild TBI) and its sequelae. **Career Development Goals:** To provide adequate time to mentor new clinical investigators and perform patient-oriented research in TBI. **Research Project:** Axonal injury is thought to be the pathophysiologic process that underlies the cognitive and motor deficits found after mild TBI. The central hypothesis of this proposal is that axonal injury results in the differential expression of serum and cerebrospinal fluid (CSF) proteins and that examination of these proteins can provide insight into the mechanism of axonal injury. The goal of this project is to examine alterations in blood and CSF to test several hypotheses related to the biochemical and cellular response to mild TBI. This goal will be accomplished through two investigative strategies. 1) Analysis of the exposure characteristic of serum and CSF proteins. 2) Correlation of protein markers of axonal damage to changes in axonal structure on diffusion tensor imaging (DTI) and cognitive function. Specifically, this project seeks to determine the putative role of a marker of neurofilament damage (pNFH) and a marker of lipid regulation (ApoA1) in axonal injury after mild TBI. The potential link between mild TBI and the increased long-term risk of neurodegeneration will be explored by an analysis of epigenetic changes in the DNA of circulating leukocytes.

To address these aims we propose to assemble a cohort of college athletes participating in contact sports in which mild TBI is a known risk, and a cohort of moderate to severe TBI patients who have an external ventriculostomy drain placed as a part of their clinical care. Because this study involves the investigation of several aspects of brain structure and function after mild, moderate and severe TBI, it serves as an ideal platform for the mentoring of new clinical investigators interested in traumatic brain injury.

This proposal meets the goals of the K24 award by 1) demonstrating the need for the PI to devote more time to and to augment his research capabilities, and by 2) providing the ideal conditions under which new clinical investigators can be mentored in the conduct of Patient-Oriented Research.

Title
Validation of Putative Serum Markers of Axonal Damage after Mild TBI

Project Narrative
Mild TBI is an important public health problem in the US for which there is currently no objective diagnostic aid and no treatment. This injury affects over 1.2 million Americans annually and is the signature injury of the conflicts in Iraq and Afghanistan. Mild TBI can result in problems thinking and performing daily activities that can last from months to years. The process by which brain cells are damaged and how this damage is linked to brain dysfunction is incompletely understood. This has prevented the development of treatments that could improve the lives of those injured. We propose to examine changes in the blood and spinal fluid to learn more about mild TBI. In the process we hope to train and inspire a new generation of clinical researchers interested in traumatic brain injury.

Facilities and Other Resources

University of Rochester Medical Center
Strong Memorial Hospital (Strong) is a 750-bed tertiary care, academic medical center, which serves as the primary teaching hospital for the University of Rochester Medical Center. It is located in the center of Monroe County. Strong serves as the regional referral center for 1.5 million residents of upstate New York. It is the regional referral center for upstate New York, providing advanced trauma, oncology, transplant, cardiovascular, pediatric, emergency, and other services. Strong Memorial Hospital is part of Strong Health, which is a health system that includes two hospitals, multiple long-term care facilities, dental facilities, home health services, and physician services. Strong is the only upstate New York hospital to be selected as one of "100 Best Hospitals in the U.S." and was the first healthcare organization in the state to receive this honor.

Emergency Department (ED)
The ED of Strong Memorial Hospital is a Regional Trauma and Burn Care Center and includes a Pediatric ED. The Department, with 43 faculty members, cares for 100,000 patients per year. The ED has the only Emergency Medicine Residency program and Pediatric Emergency Medicine Fellowship program in the region. The Emergency Medicine Residency Program is a three-year residency with 12 residents per year. The Department of Emergency Medicine has a formal relationship with the School of Medicine and Dentistry. Thus, it serves as a training site for medical students and residents from various specialties. This Department also offers fellowship training in Sports Medicine, Emergency Medical Services, and International Medicine.

Kessler Family Burn/Trauma Intensive Care Unit
The Strong Regional Burn and Trauma Center includes a 19,000-square-foot, 16- bed, world-class Burn/Trauma ICU for adults, called the Kessler Family Burn/Trauma ICU. The Kessler Family Burn/Trauma ICU is strategically located directly above the Emergency Department and is staffed 24-7 by a multidisciplinary team of experts in trauma and emergency surgery, neurosurgery, orthopedics, emergency medicine, spine surgery, vascular surgery, interventional radiology, and rehabilitation. Moderate-severe TBI patients in this unit are cared for primarily by the Neurosurgery team, with whom the Candidate has collaborated on the pilot data presented in Preliminary Studies (see Support Letter Dr. Pilcher)

Rochester Center for Brain Imaging
Concussed athlete will undergo DTI scanning at the Rochester Center for Brain Imaging (RCBI). The RCBI is a 6,000-square-foot research facility housing a Siemens 3T whole-body horizontal-bore Trio magnet, which was funded by a grant from the National Science Foundation. The Center also contains a mock magnet that can be used to train study participants before they enter the Trio. The facility resides on the ground floor of the Annex building located across from the Emergency Department of the University of Rochester Medical Center. The heart of the RCBI is a Siemens Trio 3T whole-body magnet, with maximum gradient amplitude of 40 mT/m and a slew rate 200T/m/s. A standard birdcage head coil as well as an 8 channel phased array head coil (capable of parallel imaging using SENSE/GRAPPA) are available for brain studies. Pulse sequences installed on the Trio system allow capability for many types of research applications, including functional MRI (EPI-BOLD), conventional structural MRI

(including T1 and T2 weighted and perfusion imaging), diffusion-weighted scans including diffusion tensor imaging (DTI - for imaging white matter tracts in the brain), and single- and multi-voxel spectroscopy.

University of Rochester Department of Athletics and Recreation
The University of Rochester has 22 varsity intercollegiate athletic teams that compete in NCAA Division III. The main facility for these teams is the Goergen Athletic Center, which includes the Hajim Alumni Gymnasium, Zornow Center, Field House, and Palestra. Other facilities around the River Campus include Fauver Stadium, the Peter Lyman Tennis Center, and adjacent grass playing fields. The Goergen Athletic Center features a 11,000-square-foot Fitness/Weight Facility, locker rooms, a Multi-Activity Center, an atrium, a Central Issue room for equipment, and all departmental offices. Pre and post-injury cognitive testing is done on a series of desktop computers in the athletic training room of the Goergen Athletic Center.

8B. Laboratory Environment

Center for Neural Development and Disease (CNDD)
The Bazarian Lab is located within the CNDD. The CNDD brings together faculty from diverse departments, including Neurology, Neurosurgery, Pediatrics, Biomedical Genetics, Microbiology & Anatomy, Ophthalmology, Emergency Medicine, and Neurobiology and Anatomy, to carry out research directed toward this common goal. The Center is home to six Principal Investigators (PIs), who serve as mentors to graduate students in the Interdepartmental Graduate Program in Neuroscience and other NIH-supported graduate and postgraduate training programs. Working in collaboration with research faculty that include 5 non-tenure track research assistant professors, 6 post-doctoral fellows, 17 graduate students (including M.D. Ph.D. candidates) and 24 lab technicians, technical assistants and associates, Center P.I.'s are carrying out research programs that explore a variety of issues relevant to neural development and disease. The CNDD houses suites for behavioral studies, electrophysiology and real-time microscopy rigs, climate-controlled rooms, BSL-2 hoods for vector production or other virus work, and fully equipped surgical suites for small animal procedures. The center also has facilities for animal surgery and *in vivo* multiphoton microscopy. Key pieces of equipment include ultracentrifuges, liquid nitrogen tanks for cell storage, freezers, refrigerators, and scintillation counters.

Bazarian Lab
Within the CNDD, the Bazarian Lab is comprised of two full benches, which is more than adequate to perform the work described within this proposal. Bench space contains two carrels equipped with computer drop and internet access. This lab is equipped with a temperature controlled ultra-centrifuge, microcentrifuge, water baths, balances, stir plates, pipettors, -80C and -20C freezers, orbital shaker, vortexer, full set up to run western blots and DNA gels. Shared equipment includes pH meters, chemical, cell culture and biosafety hoods, cold rooms, a fully equipped dark room (with developer), confocal microscope set up, plate reader for ELISAs, DNA gel imager, 2D gel equipment and imagers, and a full set up (instruments, rooms) for the small animal surgery.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Jeffrey J. Bazarian, MD, MPH	Associate Professor, Departments of Emergency Medicine, Neurology, Neurosurgery, Community and Preventive Medicine
eRA COMMONS USER NAME JBAZARIAN	

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Brown University, Providence RI	BA	1983	Biology
University of Rochester, School of Medicine, Rochester, NY	MD	1987	Medicine
University of Rochester Medical Center, Primary Care Program in Internal Medicine	Resident	1987-1990	Internal Medicine
University of Rochester, Rochester, NY	MPH	1999-2002	Clinical Investigation

A. Personal Statement

Dr. Bazarian in an emergency physician with an active, interdisciplinary clinical research program in mild traumatic brain injury at the University of Rochester. These efforts have been supported by a K23 from NINDS, an R01 from NICHD, as well as by funding from the New York State Department of Health and The Veteran's Administration. Dr. Bazarian has mentored over 18 junior faculty and residents, many of whom have gone on to publish their research and to obtain independent funding. In recognition of this, he was awarded the University of Rochester's Trainee Mentoring Award in 2007. These experiences and qualifications make Dr. Bazarian an ideal candidate for this K24 award.

B. Positions and Honors

Positions and Employment

1987-1990	Internal Medicine Residency, University of Rochester Medical Center, Rochester, NY
1990-1994	Emergency Physician, The Genesee Hospital, Rochester, NY.
1991-1994	Senior Clinical Instructor, University of Rochester Medical Center, Department of Medicine.
1993-1996	Senior Clinical Instructor, University of Rochester Medical Center, Department of Emergency Medicine.
1994-2001	Program Director, Rotating Residents, Strong Memorial Hospital, and Rochester NY.
1994-present	Emergency Physician, Strong Memorial Hospital, Rochester, NY.
1996-2003	Assistant Professor, University of Rochester Medical Center, Department of Emergency Medicine.
2003-present	Associate Professor, University of Rochester Medical Center, Department of Emergency Medicine.
2003-present	Associate Professor, University of Rochester Medical Center, Department of Emergency Medicine, Department of Neurology
2007-present	Associate Professor, University of Rochester Medical Center, Department of Neurosurgery
2010-present	Associate Professor, University of Rochester Medical Center, Department of Community and Preventive Medicine

Honors

1997	Excellence in Emergency Medicine Resident Clinical Teaching Award
2000	Kluge Trauma and Emergency Medical Service Award, Rochester Academy of Medicine
2003	Ernst Saward Award for Excellence in Community and Preventive Medicine
2006	Faculty Mentoring Award, University of Rochester School of Medicine

Other Experience and Professional Memberships

1990-present	Member, American College of Physicians
1997-present	Member, Society for Academic Emergency Medicine
1998-present	Member, American Association of Physician Specialists
1999-present	Fellow, American College of Physicians
2006-present	Member, American College of Emergency Physicians
2001	CDC Task Force on Mild Traumatic Brain Injury Surveillance
2003	CDC Expert Panel on TBI in Mass Trauma Events
2006	NIH/NINDS- Special Emphasis Panel: NETT Hub Review: ZNS1 SRB-K (41)
2007	NSF SBIR/STTR Disease Diagnostics and Prognostics 1 Panel: P070662
2007	Institute of Medicine, Committee on Gulf War and Health: Brain Injury in Veterans and Long Term Health Outcomes
2008	Defense and Veterans Head Injury Center Panel on Acute Care of TBI
2008	Congressionally Directed Medical Research Program, TBI/PTSD Study Section #6

C. Publications

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Preiss-Farzanegan SJ, Chapman B, Wu J, Wong T, **Bazarian JJ**. The Relationship Between Gender and Post Concussion Symptoms After Sport-Related Mild TBI. *Physical Medicine and Rehabilitation.* 2009; 1:245-253

Raun S, Noyes K, **Bazarian JJ**. Cost Effective Analysis of S-100B as a pre-head CT screen after mild TBI. *Journal of Neurotrauma* In Press

Bazarian JJ, Cernak I, Noble-Haeusslein L, Potolicchio S, Tempkin N. Long-term Neurologic Outcome after Traumatic Brain Injury. *Journal of Head Trauma Rehabilitation*. 2009;24(36):1-13. In Press

Bazarian JJ, Blyth BJ, Mookerjee S, He H, McDermott M. Sex Differences in Outcome after Mild TBI. *Journal of Neurotrauma*. In Press

D. Research Support

R01 NS41952-05 Bazarian (PI) 5/1/07 – 4/30/10
NIH/NICHD
"Detecting Axonal Damage after Mild Traumatic Brain Injury"
This study is designed to validate diffusion tensor imaging (DTI) as a measure of clinically significant axonal injury, determine the relationship between axonal injury and blood brain barrier (BBB) damage, and identify proteins unique to axonal injury after mild traumatic brain injury (TBI) using proteomic analysis of serum
Role: Principal Investigator

NYS DOH (Bazarian PI) 9/07-9/10
"Development and Validation of a Rapid Assay for serum S-100B"
In conjunction with Emergency Departments from the three other members of the Upstate Academic Health Center Consortium (Buffalo, Syracuse and Albany) this study test the ability of a rapid assay for serum S-100B to predict abnormal head CT scan after mild traumatic brain injury.
Role: Principal Investigator

Veterans Administration (Donnelly-PI) 1/1/10-1/1/11
"Cognitive Assessment of Veterans after Traumatic Brain Injury, Special Project Modification."
This study will determine the contribution of clinically defined TBI and abnormal white matter on diffusion tensor imaging to the development of post traumatic stress disorder among veterans of Operation Enduring Freedom and Operation Iraqi Freedom.
Role: Consultant

University of Rochester, Health Sciences Center for Computational Innovation (Zhong-PI) 1/1/10-1/1/11
"Highly Reliable and Efficient Diffusion Tension Imaging in a Longitudinal Study of Brain Injury"
Using mild TBI subjects, this study will develop statistical and computational methods to better compare DTI images in the same individual at two time points, and in unrelated individuals scanned on two different scanners.
Role: Co-investigator

Research Support Completed During the Last Three Years

K23 NS41952-05 Bazarian (PI) 7/1/01 – 8/31/06
NIH/NINDS
"Epidemiology of Traumatic Brain Injury"
This study investigates the relationship between detailed demographic and mechanism/location of injury data on three-month functional and cognitive outcome after mild traumatic brain injury. It also identifies injury mechanisms and geographic clusters that will aid prevention efforts.
Role: Principal Investigator

Intramural Grant, Center for Aging, University of Rochester 10/1/05-10/1/06
"Diffusion Tensor Imaging before and after Sports-related Concussion"
The goal of this study is to better define the magnitude and anatomic location of changes in DTI indices that occur after mild traumatic brain injury. This grant funds the DTI scans only and provides no salary support.
Role: Co-PI

2A. Immediate and long-term career objectives in Patient-Oriented Research and mentoring

Dr. Bazarian is currently Associate Professor in the Departments of Emergency Medicine, Neurology, Neurosurgery, and Community and Preventive Medicine at the University of Rochester Medical Center (URMC). His long-term research goal is to examine putative protein markers of traumatic AI in order to elucidate the neuropathology of mild TBI and its sequelae. Over the last 8 years, he has established a multidisciplinary team of researchers from diverse areas within URMC to investigate the process of AI after mild TBI. These scientists hail from the Departments of Emergency Medicine, Neuroscience, Neurosurgery, Neurology, Radiology, and Biostatistics.

In order to achieve these long-term goals, Dr. Bazarian has established research efforts in 3 broad areas of TBI: human neuroimaging, human serum protein analysis, and neuropathological studies of mice after controlled cortical impact (CCI). Dr. Bazarian's immediate career objectives have focused on validating DTI as a biomarker of acute AI and serum S100B as a biomarker of blood brain barrier (BBB) damage. To support his immediate and long term career objectives, Dr. Bazarian has established three patient cohorts to acquire serum, neuroimaging and clinical data from across the TBI spectrum, as well as a laboratory in the Center for Neurologic Development and Disease.

The overall purpose of better understanding the pathophysiology of AI after mild TBI is to identify potentially modifiable targets. Successful clinical trials will depend on knowledge of the process by which axonal stretch eventually leads to synaptic dysfunction, cell death and then to neuronal recovery and repair. In this regard, the current proposal is particularly important. Developing accurate biomarkers of mild TBI is not only a fundamental step toward understanding the natural history of mild TBI and its sequelae, these markers can provide invaluable clues into the underlying pathophysiology of AI, and insight into future diagnosis and treatment. The Candidate would like his research program to lead the way in understanding the process of AI after mild TBI in humans. In the process, he hopes to train and inspire a new generation of clinicians to be independent researchers within the field of neurotrauma.

2B. Summary of Candidate's Research Career

After residency training, Dr. Bazarian completed the Masters of Public Health-Clinical Investigator track under the mentorship of Thomas Pearson, MD, MPH, PhD, who is Associate Dean for Clinical Research at URMC. In 2001 Dr. Bazarian was one of the first emergency physicians nationally to be awarded a 5-year Career Development Award (K23) from the NINDS. The focus of his research was TBI epidemiology and outcomes. During the K23, Dr. Bazarian's research interest shifted to identifying and elucidating the process of AI after TBI, especially after mild TBI. Mild TBI is an injury commonly encountered in the emergency setting for which there is no objective diagnosis and no treatment. He assembled a diverse group of researchers within the University of Rochester to address this problem. He was awarded an R01 from the NICHD in 2007 to investigate DTI as an imaging marker of AI and to gain insights into the process of AI by examining changes to the serum proteome. This award has allowed Dr. Bazarian to establish an active laboratory within the Center for Neural Development and Disease, to collaborate with other neuroscientists in that Center, and to assemble several human TBI cohorts. This translational arrangement has attracted a host of trainees interested in studying the pathophysiology and sequelae of TBI. The K24 award would give Dr. Bazarian the protected time to formally mentor new clinical investigators while simultaneously

allowing him to devote more time to and to augment his capabilities in Patient-Oriented Research.

Dr. Bazarian has served on several national TBI-related task forces and panels for the Centers for Disease Control and Prevention, the NIH, the National Science Foundation, and the Institute of Medicine. He has participated in three Defense and Veterans Head Injury Center panels to develop guidelines for the diagnosis and treatment of mild TBI among US forces injured in Iraq and Afghanistan. In addition, he was an active member of an Institute of Medicine panel that presented a Report to Congress detailing the long term health effects of TBI, including seizures and Alzheimer's Disease.[1, 2] These activities have put the Candidate in contact with the nation's leading TBI researchers, enhancing the quality of both his patient oriented research and his mentoring.

2C. Evidence of Ongoing High Quality Patient Oriented Research

Current Patient-Oriented Research The candidate's current Patient-Oriented research falls into 3 areas:

Using DTI to gain insight into AI after mild TBI. Evaluated changes in DTI indices over 3 time points after mild TBI as part of his R01 award, "Detecting Axonal Damage after Mild TBI".

Understanding the relationship between BBB and traumatic AI. Determined the relationship between BBB damage and the temporal pattern of release of serum S100B, phosphorylated neurofilament heavy (pNFH) chain and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) into serum among moderate-severe TBI patients. (see Preliminary Studies)

Examining mild TBI-related changes in the serum proteome. Identified two proteins upregulated after mild TBI group: Apoprotein A1 and serpin. (see Preliminary Studies)

Proposed Patient-Oriented Research The candidate is involved in several projects that support the proposed patient oriented research. (1) Assembling a cohort of student athletes and piloting the collection of pre and post-injury serum and cognitive data. (see Preliminary Studies) (2) Assembling a cohort of moderate-severe TBI subjects and piloting the collection of paired CSF and serum samples. (see Preliminary Studies) (3) Working with the University of Rochester's Institutional Review Board to develop a protocol for the collection of circulation leukocytes from humans for the purposes of DNA analysis.

Evidence of Monetary Support for Patient-Oriented Research The Candidate's research has enjoyed a stable funding stream as summarized in **Table 1**.The Candidate's current R01 is supported by 4 more months of funding. However, a no-cost extension should allow R01 activities to progress for an additional 9-12

Table 1: Candidate's Monetary Support for Patient Oriented Research

Year	Role	Funding Agency	Grant Title
1996-7	PI	Emergency Medicine Foundation	Cognitive Predictors of Outcome after Mild TBI
2001-06	PI	NINDS K23	Epidemiology of Mild TBI
2005	PI	URMC Center for Aging	DTI after Mild TBI: A Pilot Study
2005-6	PI	URMC Center for Aging	DTI Before and After Sports-Related Concussion
2007-10	PI	NYS Department of Health	Development and Validation of a Rapid Assay for Serum S100B
2007-10	PI	NICHD R01	Detecting Axonal Damage After Mild TBI
2010-11	Consultant	Veterans Administration	Cognitive Assessment of Veterans after Traumatic Brain Injury, Special Project Modification

months. The Candidate plans to explore the long term fate of axonally injured brain in these subjects as part of a competitive renewal of this R01, which will be submitted in July 2010. These Patient-Oriented Research experiences have allowed the candidate to grow and develop into an effective and accessible mentor, as described below.

2D. Candidate's Commitment to a Career in Patient-Oriented Research

After residency, the candidate was inspired to pursue research training by the work of Rochester Neurologist Mary Dombovy, who was analyzing the effects of amandatine on post-TBI cognitive deficits. During his MPH training, the candidate applied clinical research methods to the study mild TBI, which had recently been declared a public health threat by the NIH.[3] The candidate developed a unique feel for the size of the public health problem posed by mild TBI in the urban academic emergency department (ED) in which he actively worked as faculty in the Department of Emergency Medicine. There, he would typically see 5-10 concussion patients a day. From this experience and from his subsequent K23 work, the candidate observed that large knowledge gaps in nature of mild TBI was a fundamental obstacle to developing treatments. To address these issues, the candidate created a TBI research program at the University of Rochester where one did not previously exist. He assembled a diverse team of students, residents, and scientists from several departments who are all now focused on neuroimaging and protein analysis after mild TBI. However for the science to continue to move forward—to a better understanding of the natural history of AI and how to limit its deleterious effect on brain function--the team needs more than one clinical researcher. New clinical investigators are sorely needed to accomplish these goals. The candidate's genuine passion for teaching, combined with his desire to see new clinicians take an interest in TBI research, makes him an ideal candidate for this K24 award.

2E. Ability of Candidate to Provide Mentoring to New Clinical Investigators

Dr. Bazarian has considerable prior experience as a mentor for over 18 junior faculty and residents. (See **Table 2** for recent mentees) Many of these mentees have gone on publish their research and to obtain independent funding. For example, after the candidate's mentorship, Dr. Brian Blyth (**Table 2**), was promoted to Assistant Professor of Emergency Medicine and was awarded the Society for Academic Emergency Medicine's (SAEM) Research Training Grant (2004) giving him 2 years of salary support. He has authored 5 publications since 2007. Dr. Lynn Cimpello's mentorship by the Candidate directly led to her being awarded the SAEM's Research Training Grant. In addition, in 2005 she was award a grant from Emergency Medical Services for Children / Maternal and Child Health Bureau of HRSA to study pediatric TBI. She is currently the director of Pediatric TBI research at the University of Cincinnati Children's Hospital. Success stories such as these earned Dr. Bazarian the University of Rochester's Trainee Mentoring Award in 2007. (see Appendix) This commitment to mentoring makes him an ideal candidate for guiding new clinical investigators in the conduct of Patient-Oriented Research. This K24 award would give Dr. Bazarian the protected time to formally mentor new clinical investigators while simultaneously allowing him to devote more time to and to augment his capabilities in Patient-Oriented Research.

Table 2: Dr. Bazarian's Most Recent Mentees and Their Accomplishments

Name	Career Stage Mentored	Research Mentored	Accomplishment	Current Academic Position
B.Blyth MD	EM Jr Faculty	TTR and S-100B as markers of BBB damage	RWJ Grant Publication	Assistant Professor of EM, URMC

L Cimpello MD	EM Jr. Faculty	Serum Markers of TBI in Preverbal Children	Publication SAEM Grant	Assistant Prof of EM and Peds, Dir.of Peds TBI Research, U Cincinnati Children's Hospital
S Ruan	MD-PhD student	Cost effectiveness of S-100B	Publication	MD-PhD student
V Stocklein	Medical Student	TTR and S-100B as markers of BBB damage	Publication	Medical Student Ludwig Maximillian University, Munich
H Adams PhD	Post-doc fellow in Child Psychiatry	Deficit-specific education for pediatric Mild TBI	URMC General Clinical Research Center-CREFF Award	Assistant Professor of Neurology and Pediatrics
M Garcia MD	Pediatric EM Fellow, Jr Faculty	ATV-Related TBIs in the US, CT imaging of pediatric appendicitis.	Manuscript PECARN grant	Assitant Professor EM, Pediatrics URMC
M Badawy MD	Pediatric EM Fellow, Jr Faculty	Dev and Validation of a Post-TBI Tel Outcome Instrument for Children	Ken Graff Award Young Investigator Award/AAP	Interim Director of Peds EM Fellowship, URMC

2F. Protected Time Will Contribute to Candidate's Research Program and Mentoring

Dr. Bazarian's current clinical, teaching, administrative and research responsibilities are given in **Table 3** below. Dr. Bazarian's prior mentorship activities were performed primarily on his free time and at the expense of his research productivity. This approach to mentoring is counterproductive for his long-term research career. The K24 award would, in fact, propel his career trajectory by giving him protected time to mentor new clinical researchers while simultaneously expanding his research capabilities. Dr. Bazarian's Department Chair is committed to relieving him of clinical and administrative duties that would allow him to commit 50% effort to this award during all 5 yeas of the award period. (see Institutional Commitment letters)

Table 3 Candidate's Current Responsibilities

Responsibility	Approximate Percent Effort
Administrative/Teaching	
Committees, resident lectures	5%
Clinical	
12 hrs/week in Emergency Department	25%
Research	
R01 Award	60%-ends April 2010
NYS Department of Health Award	10%-ends Sept 2010

3A. Candidate's Career Goals and Objectives The candidate's career development goals are to provide adequate time to mentor new clinical investigators and to perform patient-oriented research in TBI. Currently there are no standard diagnostic tools to detect AI. This has frustrated efforts to understand the natural history of AI in humans and its relationship to clinical recovery. The candidate's research goals are to understand the pathophysiology of AI in vivo in humans through analysis of brain-related proteins in serum and CSF. His currently funded research has focused on imaging AI acutely using DTI. A planned competitive renewal of this R01 (July 2010) will focus on analyzing the relationship between the acute changes to the axon and the long-term fate of these structures as seen on DTI. The proposed project attempts to better understand the cellular response to AI by analyzing proteins related to neurofilament damage and lipid regulation.

3B. Prior Experience The candidate's prior patient-oriented research falls into 3 areas:
Epidemiology of Mild TBI. Determined predictors of outcome after mild TBI using cognitive testing done in the ED, determined the epidemiology of and treatments administered to mild TBI patients presenting to a national sample of EDs, estimated the effect of impact direction on the risk of TBI during car crashes, determined the epidemiology of sports-related mild TBI, and determined the influence of gender on outcome after mild TBI.
DTI as a biomarker of clinically significant AI. Detected significant changes in the directionality and magnitude of water diffusion in the white matter after mild TBI patients using DTI, and refined the statistical methods by which these indices are analyzed and understood.
BBB dysfunction after TBI. Examined the accuracy of serum S-100B for predicting abnormal BBB, and examined the relationship between S100B to hemorrhage on head CT scan.

3C. Current Research Support Dr. Bazarian's current research support is given in **Table 4** below.

Table 4 Dr. Bazarian's Current Research Support

Year	Role	Funding Agency	Grant Title
2007-2010	PI	NYS Department of Health	Development and Validation of a Rapid Assay for Serum S100B
2007-2010	PI	NICHD R01	Detecting Axonal Damage After Mild TBI
2010-2011	Consultant	Veterans Administration	Cognitive Assessment of Veterans after Traumatic Brain Injury, Special Project Modification

3D. Timeline for Accomplishing Career Goals

Table 5 Career Development Timeline

Activity	Year 1	Year 2	Year 3	Year 4	Year 5
Mentor Development through URMC CTSA					
Submit competitive renewal of current R01	July 2010				
Carry out renewed R01					
Proposed K24 Study					
Subject accrual					
Analysis					
New hypotheses regarding mechanisms of axonal injury					
Submit new R01					
Mentoring New Clinical Investigators	Successfully Mentor 5-7 Trainees Over 5 Years				

The candidate expects to accomplish several career-related activities during the award period, as summarized in **Table 5**. Research related activities will serve as the platform on which to mentor 5-7 trainees over a 5 year period. In addition it is hoped that these activities will generate hypotheses that will form the basis for a new R01 application.

Continued Mentorship Development In order to provide the best mentorship possible, the candidate strives to continue to improve his teaching techniques and to understand new concepts in learning. The candidate will participate in the Mentor Development Core (MDC) supported by the URMC Clinical and Translational Science Award (CTSA), which is designed to promote and sustain mentor development. The MDC has developed a flexible set of activities including a structured mentor-protégé curriculum, faculty development seminar series, campus-wide workshops, development of "best practices", and maintenance of a mentor pool. The MDC provides interval meetings with a senior clinical researcher to guide the mentor-mentee relationship. Finally, the candidate will attend the weekly Rochester Clinical & Translational Research Curriculum Seminar Series which brings in guest speakers to discuss various aspects of clinical and translation research. The URMC CTSA will be renewed in July, 2011. The renewal application is due June 1, 2010 with 12 institutions applying for 12 renewed awards. The University has a strong CTSA Program and expects to successfully renew the CTSA so it will be active throughout the period of funding of the K24. (see Institutional Support, Dr. Pearson's letter)

Patient-Oriented Research in Mild TBI The candidate will participate in currently funded research activities related to mild TBI as well as in the research project proposed here. This project will augment the research capabilities of the candidate and serve as a platform on which to mentor new clinical investigators

Mentoring New Clinical Investigators The training of new clinical investigators is an activity that will contribute to the career development of the candidate. The candidate hopes to mentor 5-7 new clinical investigators over the 5 year award period.

Program to Provide Instruction in the Responsible Conduct of Research All mentees will undergo training in the responsible conduct of research prior to performing any research activities. This will be accomplished by participating in the Human Subjects Protection Program, sponsored by the University of Rochester Human Subjects Review Board. This is a self-study program consisting of an informational textbook and an examination. The textbook [Dunn, C.M., & Chadwick, G.L. (2004). Protecting Study Volunteers In Research: A Manual for Investigative Sites (3rd ed.). Boston: CenterWatch, Inc.] was developed by University of Rochester faculty and staff and reviewed by an external (national) advisory committee. In addition all mentees will participate in one of the following two University of Rochester courses: IND 503 Ethics in Research or IND 501 Ethics and Professional Integrity. These courses are designed to enable attendees to appreciate the importance of applying ethical principles to the conduct of biomedical research, to understand the basic framework of guidelines, rules and regulations which exist to assess ethical issues arising in biomedical research and to acquire a set of basic biomedical ethics resources to apply to biomedical research. Topics reviewed include conflict of interest, responsible authorship, policies for handling misconduct, policies for the use of human and animal studies, and data management.

6A. Availability of New Clinical Investigators for Mentoring

New clinical investigators will come from a variety of sources. The first and perhaps most fruitful source will be within the candidate's own Department of Emergency Medicine. Junior faculty and faculty just out of residency training will most likely be in need of research training. Because fellowship programs involving formal research training are only now starting to develop within the specialty of Emergency Medicine, most new faculty come to work in an academic environment without prior training. As can be seen in Table 2, many of Dr. Bazarian's prior mentees have been junior faculty in Emergency Medicine. Fellows participating in one of the four fellowship programs offered by the Department of Emergency Medicine (Pediatric Emergency Medicine, Emergency Medical Services, International Medicine, and Sports Medicine) could also be potential trainees.

However, new clinical investigators will also come from residents and fellows from other disciplines. As of 2009, URMC had 536 ACGME-accredited residency positions across 25 programs, and 146 fellowship positions across 50 programs. Residency programs in surgery and neurosurgery have a non-ACGME year(s) for research; orthopedic surgery has an ACGME-approved research year for one resident. Two neurosurgery residents are currently engaged in TBI research. (see Support Letter from Dr. Pilcher) Most URMC fellowship positions have a research expectation as a core requirement. The Department of Neurology, for example, offers fellowship training in several areas that overlap with neurotrauma including Experimental Therapeutics. (see Support Letter from Dr. Griggs) In addition, there are 25 T32 training programs providing for 98 pre-doctoral positions and 64 post-doctoral positions and 5 R25 awards. Of the 25 T32 programs, 12 are in clinical departments. All are potential sources of mentees.

In addition, new investigators can also come from schools within University of Rochester that provide both professional and research training in their respective fields. These programs span from undergraduate students to advanced training degrees in each of the specialty areas. During the 2009-2010 academic year the School of Medicine had 61 students enrolled in its MD/PhD Program, the School of Nursing had 35 students enrolled in its PhD program, and the School of Liberal Arts and Science had 67 students enrolled as Rochester Early Medical Scholars. The School of Medicine additionally encourages its medical students to spend a year between 2nd and 3rd year doing research as part of the Academic Research Track. Several students have already expressed in interest in studying TBI. (see Support Letter Dr. Gross) The Department of Community and Preventive Medicine offers several research degree programs (MPH-73 current students, MS-Clinical Investigation 17 students, PhD-34 students), and has a programmatic interest in TBI epidemiology and prevention. (see Support Letter Dr. Fisher) These Letters of Support underscore the appeal the proposed training program would have to a variety of trainees.

Finally, the CTSA has attracted a variety of health care professional to training in patient oriented research. The CTSA Education Training and Career Development Key Function in particular has generated new clinical researchers in need of mentorship. The two new Masters Programs in Translational Research and in Clinical Investigation have growing numbers of students enrolled. A supplemental grant to the Rochester CTSA has been received to develop the National CTSA Educational Resource Center at URMC, consisting of a virtual network of mentors for new clinical investigators from the 38 CTSA institutions. Finally, the Upstate New York Translational Research Network, supported by the Rochester CTSA and consisting of 13 institutions, also provides mentorship to biomedical researchers from across Upstate New York.

6B. Educational and Research Experiences That Will be Provided to New Clinical Investigators

Didactic Courses Mentees will be encouraged to take 1-2 didactic courses to strengthen their fund of knowledge in the process of clinical research and the science of traumatic brain injury. **Table 6** shows a partial listing of the courses offered. These courses are drawn from the several degree programs within the University (MPH, MS in Clinical Investigation, MS in Translational Research and PhD in Translational Biomedical Science).

Table 6 Didactic Courses Available to New Clinical Investigators

Course Title	Credits	Course Title	Credits
Introduction to Biostatistics	4	Laboratory Methods for Translational Research	3
Advanced Biostatistics	4	Intro to Data Management Using SAS	4
Statistical Methods for Biomedical Applications	4	Design of Clinical Trials	3
Ethics in Research	1	Survey Research	4
Introduction to Epidemiology	3	Cost Effectiveness Research	4
Advanced Epidemiologic Methods	3	Introduction to Health Informatics	2
Epidemiologic Methods	4	Introduction to Clinical Research	3
Molecular Epidemiology	3	Biology of Neurologic Diseases	4
Field Epidemiology	3	Integrated Systems Neuroscience	4
Practical Skills in Grant Writing	3		

Workshops

Mentees will be encouraged to attend one of the following two workshops available at the University of Rochester.

Writing workshop: This two-part course helps students gain proficiency as writers. The course addresses language usage, outlines, quotations, transitions and the use of sources. The course will also help mentees eliminate jargon, advance an argument effectively, and develop skills to focus on audience, message and purpose.

Workshop in scientific communication: This workshop will address the principle elements of scientific presentation and communication such as the preparation of abstracts and journal articles, poster development, manuscript review and critique, oral presentations, and working with the media/public relations.

The following workshops are offered through the URMC CTSA. Mentees will be encouraged to attend at least one of the following:

Research Administration Skill Building workshop
Workshop on Technology Transfer
Workshop on Retention and Recruitment of Human Subjects in Clinical Trials
Workshop on Community Based Participatory Research

Lecture Series

The weekly Rochester Clinical Translational Research Curriculum includes presentations from UR training mentors, guest lecturers, experts in technological innovations in clinical research, as well as from trainees**.** Mentees will be encouraged to attend one lecture per month.

Research Experience

The Candidate will provide the mentee with the skills necessary to carry out clinical research. For the proposed study, mentees will be taught how to obtain informed consent form from subjects, how to administer a computerized cognitive test, and how to draw blood from subjects. They will learn how to organize, process, and store serum samples. Mentees will undergo MRI safety training and will first observe and then participate in the DTI scanning of subjects. Mentees will be instructed in data entry and retrieval using common data processing programs. Finally, they will be instructed in basic laboratory techniques included Western blotting and gel electrophoresis.

The candidate can also provide mentees with additional skills in patient oriented research. These include analysis and interpretation of DTI images, analysis and interpretation of computerized cognitive testing, performance and interpretation of postural stability testing, and DNA analysis. The candidate can provide mentees with the skills necessary to obtain serum from arterial catheters and CSF from external ventriculostomy drains in severely injured TBI patients. Finally, mentees will have access to veterans of Operations Iraqi Freedom and Enduring Freedom who have suffered blast-related TBI and are undergoing DTI imaging as part of cooperative study with the Veterans Administration Hospital in Buffalo, NY. These skills and patient care opportunities should appeal to a wide range of mentees.

Mentees will be required to attend weekly meetings of the TBI team in which data accumulated from the prior week are reviewed and activities for the upcoming week planned. Mentees will be encouraged to participate in all discussions. They will also be required to give a formal 20-30 minute didactic presentation to the TBI team on an aspect of TBI that is of interest to them. The candidate will strongly encourage the mentee to develop an independent research project. Ideas for this project may come from the mentee's prior experience, from research done with the candidate, or from ideas generated during TBI team meetings. Mentees will be required to present their research ideas to the TBI team and integrate feedback into the design. The candidate will supervise the drafting of a research proposal, and the submission of that proposal to the institutional research subjects review board. Upon completion of the independent research project, mentees will present their results in the form of an oral abstract presentation to the TBI research team (and perhaps at a scientific meeting) and to work closely with the mentor to put the research results in manuscript form.

Developing an Individualize Academic Development Plan

Although this is the last item listed in this section, it is likely the most important and is intended to be carried out at the beginning of the mentee-mentor relationship, before any courses are taken or research activities begun. The candidate will work closely with the mentee to craft an individual academic development plan. The process is quite simple and starts with the mentee articulating his/her academic career goals in four important areas: fundamental research knowledge or skills, specific research accomplishments, teaching and communication skills, and professional development. The mentor helps the mentee decide the best way to achieve each goal and a deadline for completing these tasks is set. Finally, a means by which both the mentor and mentee can verify that the goal was reached must be explicitly articulated. Mentees will be required to fill out a form based on **Table 7** upon beginning with the mentor. This Table was adapted from one provided by the URMC CTSA as part of the *Mentoring the Mentor* lecture series.

Table 7 Academic Development Plan for New Clinical Investigators

Mentee Academic Development Plan				
Fundamental Research Knowledge or Skills				
Long Term Goals (Describe what you need to learn to position yourself academically in 5-10 years)				
• Goal 1				
• Goal 2				
Specific Implementation Method to attain long term needs	Date of start of activity	Date of completion	Means of verification	Comments and concerns
• Method 1				
• Method 2				
Specific Research Accomplishments				
Long Term Goals (Describe what you need to learn to position yourself academically in 5-10 years)				
• Goal 1				
• Goal 2				

Specific goals to meet long term needs	Date of start of activity	Date of completion	Means of verification	Comments and concerns
• Method 1				
• Method 2				
Teaching and Communication Skills				
Long Term Goals (Describe what you need to learn to position yourself academically in 5-10 years)				
• Goal 1				
• Goal 2				
Specific goals to meet long term needs	Date of start of activity	Date of completion	Means of verification	Comments and concerns
• Method 1				
• Method 2				
Professional Development				
Long Term Goals (Describe what you need to learn to position yourself academically in 5-10 years)				
• Goal 1				
• Goal 2				
Specific goals to meet long term needs	Date of start of activity	Date of completion	Means of verification	Comments and concerns
• Method 1				
• Method 2				

Mentees will meet with the candidate one-on-one to review and update the development plan 2-4 times a month, although the frequency of these meetings will likely vary based on the mentee's clinical responsibilities. The candidate anticipates devoting 20% effort to mentoring during all five years of the award period.

6C. Plan for Supporting the Research of Mentees During the Award Period.

Financial support for mentees during the award period can come from a number of sources. Junior faculty will be encouraged to apply for either a KL2 award through the URMC CTSA or for a K23 from the NIH. Since the candidate completed a K23 award in 2006 and participates in the KL2 grant review committee for the URMC CTSA, he is in a good position to advise mentees on these awards. Junior faculty and fellows in Emergency Medicine are eligible to apply for a 2-year research training grant from the Emergency Medicine Foundation / Society for Academic Emergency Medicine. Medical students enrolled in the Academic Research Track are supported in full by the CTSA and Medical School during the research year. Neurosurgery and orthopedic residents required to do one year of research are supported in full by their department. Medical students can apply for 3 months of research training from Emergency Medicine Foundation / Society for Academic Emergency Medicine, or one year of support from the URMC Academic Research Track. (see Dr. Pearson's Letter for Institutional Support)

Facilities and Other Resources Available to the Candidate

8A. Clinical Environment

University of Rochester Medical Center
Strong Memorial Hospital (Strong) is a 750-bed tertiary care, academic medical center, which serves as the primary teaching hospital for the University of Rochester Medical Center. It is located in the center of Monroe County. Strong serves as the regional referral center for 1.5 million residents of upstate New York. It is the regional referral center for upstate New York, providing advanced trauma, oncology, transplant, cardiovascular, pediatric, emergency, and other services. Strong Memorial Hospital is part of Strong Health, which is a health system that includes two hospitals, multiple long-term care facilities, dental facilities, home health services, and physician services. Strong is the only upstate New York hospital to be selected as one of "100 Best Hospitals in the U.S." and was the first healthcare organization in the state to receive this honor.

Emergency Department (ED)
The ED of Strong Memorial Hospital is a Regional Trauma and Burn Care Center and includes a Pediatric ED. The Department, with 43 faculty members, cares for 100,000 patients per year. The ED has the only Emergency Medicine Residency program and Pediatric Emergency Medicine Fellowship program in the region. The Emergency Medicine Residency Program is a three-year residency with 12 residents per year. The Department of Emergency Medicine has a formal relationship with the School of Medicine and Dentistry. Thus, it serves as a training site for medical students and residents from various specialties. This Department also offers fellowship training in Sports Medicine, Emergency Medical Services, and International Medicine.

Kessler Family Burn/Trauma Intensive Care Unit
The Strong Regional Burn and Trauma Center includes a 19,000-square-foot, 16- bed, world-class Burn/Trauma ICU for adults, called the Kessler Family Burn/Trauma ICU. The Kessler Family Burn/Trauma ICU is strategically located directly above the Emergency Department and is staffed 24-7 by a multidisciplinary team of experts in trauma and emergency surgery, neurosurgery, orthopedics, emergency medicine, spine surgery, vascular surgery, interventional radiology, and rehabilitation. Moderate-severe TBI patients in this unit are cared for primarily by the Neurosurgery team, with whom the Candidate has collaborated on the pilot data presented in Preliminary Studies (see Support Letter Dr. Pilcher)

Rochester Center for Brain Imaging
Concussed athlete will undergo DTI scanning at the Rochester Center for Brain Imaging (RCBI). The RCBI is a 6,000-square-foot research facility housing a Siemens 3T whole-body horizontal-bore Trio magnet, which was funded by a grant from the National Science Foundation. The Center also contains a mock magnet that can be used to train study participants before they enter the Trio. The facility resides on the ground floor of the Annex building located across from the Emergency Department of the University of Rochester Medical Center. The heart of the RCBI is a Siemens Trio 3T whole-body magnet, with maximum gradient amplitude of 40 mT/m and a slew rate 200T/m/s. A standard birdcage head coil as well as an 8 channel phased array head coil (capable of parallel imaging using SENSE/GRAPPA) are available for brain studies. Pulse

sequences installed on the Trio system allow capability for many types of research applications, including functional MRI (EPI-BOLD), conventional structural MRI (including T1 and T2 weighted and perfusion imaging), diffusion-weighted scans including diffusion tensor imaging (DTI - for imaging white matter tracts in the brain), and single- and multi-voxel spectroscopy.

University of Rochester Department of Athletics and Recreation
The University of Rochester has 22 varsity intercollegiate athletic teams that compete in NCAA Division III. The main facility for these teams is the Goergen Athletic Center, which includes the Hajim Alumni Gymnasium, Zornow Center, Field House, and Palestra. Other facilities around the River Campus include Fauver Stadium, the Peter Lyman Tennis Center, and adjacent grass playing fields. The Goergen Athletic Center features a 11,000-square-foot Fitness/Weight Facility, locker rooms, a Multi-Activity Center, an atrium, a Central Issue room for equipment, and all departmental offices. Pre and post-injury cognitive testing is done on a series of desktop computers in the athletic training room of the Goergen Athletic Center.

8B. Laboratory Environment

Center for Neural Development and Disease (CNDD)
The Bazarian Lab is located within the CNDD. The CNDD brings together faculty from diverse departments, including Neurology, Neurosurgery, Pediatrics, Biomedical Genetics, Microbiology & Anatomy, Ophthalmology, Emergency Medicine, and Neurobiology and Anatomy, to carry out research directed toward this common goal. The Center is home to six Principal Investigators (PIs), who serve as mentors to graduate students in the Interdepartmental Graduate Program in Neuroscience and other NIH-supported graduate and postgraduate training programs. Working in collaboration with research faculty that include 5 non-tenure track research assistant professors, 6 post-doctoral fellows, 17 graduate students (including M.D. Ph.D. candidates) and 24 lab technicians, technical assistants and associates, Center P.I.'s are carrying out research programs that explore a variety of issues relevant to neural development and disease. The CNDD houses suites for behavioral studies, electrophysiology and real-time microscopy rigs, climate-controlled rooms, BSL-2 hoods for vector production or other virus work, and fully equipped surgical suites for small animal procedures. The center also has facilities for animal surgery and *in vivo* multiphoton microscopy. Key pieces of equipment include ultracentrifuges, liquid nitrogen tanks for cell storage, freezers, refrigerators, and scintillation counters.

Bazarian Lab
Within the CNDD, the Bazarian Lab is comprised of two full benches, which is more than adequate to perform the work described within this proposal. Bench space contains two carrels equipped with computer drop and internet access. This lab is equipped with a temperature controlled ultra-centrifuge, microcentrifuge, water baths, balances, stir plates, pipettors, -80C and -20C freezers, orbital shaker, vortexer, full set up to run western blots and DNA gels. Shared equipment includes pH meters, chemical, cell culture and biosafety hoods, cold rooms, a fully equipped dark room (with developer), confocal microscope set up, plate reader for ELISAs, DNA gel imager, 2D gel equipment and imagers, and a full set up (instruments, rooms) for the small animal surgery.

8C. Mentoring Environment

Clinical and Translations Research Award (CTSA)

The University of Rochester was one of the first 12 CTSAs to be awarded by NIH in 2006. The CTSA currently has five different pilot programs and 12 consultation services to support research and trainees. One of the largest components of the URMC CTSA is Education, Training, and Career Development. New clinical investigators have a variety of support mechanism available. For example, the Academic Research track supports medical students to take a year out doing research. The KL2 Research Development Award funds junior faculty at 75% effort for 3-5 years to participate in patient-oriented research. The CTSA also supports the capabilities of mentors. Regular faculty development workshops provide ongoing mentor development and interval meetings with a senior clinical researcher are available to guide the mentor-mentee relationship. A weekly Rochester Clinical & Translational Research Curriculum Seminar Series brings in guest speakers to discuss various aspects of clinical and translation research. In 2011, a 200,000 square foot Clinical and Translational Science Building will be completed to support patient-oriented research. The URMC CTSA will be renewed in July, 2011. The renewal application is due June 1, 2010 with 12 institutions applying for 12 renewed awards. The University has a strong CTSA Program and expects to successfully renew the CTSA so it will be active throughout the period of funding of the K24. (see Institutional Support, Dr. Pearson's letter)

Institutional K30

The Clinical Research Curriculum (K30) is integrally linked with a Master's Degree in Translational Research, in Clinical Investigation, and in Public Health, and a PhD program in Translational Biomedical Science. These degree programs, as well as the courses in them, are available to new clinical investigators.

TBI Research Team

The TBI research team meets weekly in the CNDD. Teams members include the candidate, several other faculty-level TBI researchers, lab technicians, patient coordinators, and medical students and residents currently performing TBI research. This team provides an ideal environment to provide additional guidance to new clinical investigators in ways that a single mentor could not. Team members are available to provide feedback on manuscripts and abstract presentations.

10. Specific Aims

The Candidate's currently supported research has focused on gaining insight into the process of AI after mild TBI through the use of diffusion tensor imaging (DTI) and longitudinal changes in the BBB. The key underlying assumption of this research is that AI is the structural substrate behind post-mild TBI neurologic dysfunction, and that axonal swelling is detectable with DTI. This research, and in particular the R01 ("Detecting Axonal Damage after Mild TBI"), has generated new hypothese that the Candidate seeks to test in the current proposal.

The overarching hypothesis of this proposal is that AI results in the differential expression of serum and CSF proteins and that examination of these proteins can provide further insight into the mechanism of axonal injury. The primary goal of this K24 project, thus, is to examine alterations in blood and CSF to test several hypotheses related to the biochemical and cellular response to mild TBI. This goal will be accomplished through two investigative strategies. 1) Analysis of the exposure characteristic of serum and CSF proteins related to AI (pNFH) and recovery from AI (Apolipoprotein A1) 2) Correlation of protein markers of axonal damage (pNFH) to changes in axonal structure on DTI and cognitive function. Finally, the potential link between mild TBI and the increased long-term risk of neurodegeneration will be explored by an analysis of epigenetic changes in the DNA of circulating leukocytes. A cohort of college athletes participating in contact sports in which mild TBI is a known, and a cohort of moderate to severe TBI patients who have an external ventricular drain placed as part of their clinical care will be assembled to accomplish these goals.

The Specific Aims of this study are thus to:

Specific Aim 1: Determine the putative role of pNFH after mild TBI.

Hypothesis: *pNFH is a marker of early axonal swelling*. Serum pNFH levels will be measured pre and at 4 time points post-mild TBI and correlated to decreases in fractional anisotropy (FA) on DTI.

Hypothesis*: pNFH diffusion from CSF to serum does not depend on BBB permeability.* The ratio of pNFH in serum to pNFH in CSF will be correlated to the albumin quotient, (the gold standard for measuring BBB permeability) at 3 time points after moderate to severe TBI

Specific Aim 2: Determine the putative role of Apolipoprotein A1 (ApoA1) after mild TBI.

Hypothesis**:** *Peripheral increases in ApoA1 represent an adaptive, systemic response to axonal injury.* Changes in serum ApoA1 levels after mild TBI will be correlated to changes in cognitive performance.

Hypothesis: *ApoA1 diffuses from the serum and into the CSF in response to TBI.* CSF and serum ApoA1 levels will be measured at 3 time points after mod-severe TBI and compared to the albumin quotient.

Specific Aim 3: Analyze leukocyte DNA for global epigenetic changes before and after mild TBI.

Hypothesis: *Axonal injury is associated with alterations in DNA methylation and histone acetlyation patterns, providing a potential mechanistic link between mild TBI and neurodegeneration.* Compared to pre-injury, the post-injury DNA of injured athletes will show decreased methylation and histone acetylation.

This study will serve as an ideal platform for the mentoring of new clinical investigators. Mentees will have the unique opportunity to interface with mild, moderate, and severe TBI subjects, to obtain clinical samples from them, and to analyze these samples at the bench. In addition, they will gain experience in cognitive testing and the performance and interpenetration DTI images. Mentees will witness first-hand the effects of mild through severe TBI on several aspects on brain structure and function and experience a truly translational experience. The candidate anticipates devoting 30% effort to this research project during all five years of the award.

11A. Significance

Mild TBI and AI Mild TBI affects nearly 1.2 million Americans annually[4], as well as US soldiers involved in combat operations in Iraq and Afghanistan, and civilians who survive terrorist bombings. Despite the designation 'mild', this injury causes post concussive symptoms and cognitive deficits lasting more than a year in 25%.[5] There are no known treatments. The diagnosis of mild TBI relies on symptoms and signs without any objective diagnostic aids. The lack of an accurate and objective method to diagnosis mild TBI is an obstacle to the development of potential therapies. The most appropriate target for such an objective diagnostic aid is axonal injury (AI). A key underlying assumption of the current proposal is that AI is the structural substrate behind post-mild TBI neurologic dysfunction. Studies in animals and humans have revealed AI after all severities of TBI, including mild TBI.[6] During AI, stretch-induced calcium influx leads to axonal microtubule and neurofilament destruction and disruption of axonal transport leading to axonal swelling.[7, 8] These events provide the opportunity to detect AI in humans. Disruption of axonal transport leading to axonal swelling can be visualized by DTI.[9] Microtubule and neurofilament destruction release a variety of proteins, which may be detectable in serum if the BBB is damaged.[10] The mechanism, natural history and recovery from AI are incompletely understood. These fundamental questions can be addressed when a reliable method for detecting AI in humans has been identified. The Candidate proposes to use DTI to evaluate serum pNFH as a marker of axonal damage and serum ApoA1 as playing a role in recovery from this damage.

DTI as a Biomarker of Axonal Injury DTI can detect the changes in brain water movement thought to accompany AI. DTI measures the nonlinear movement of water in 6 or more noncollinear directions. This allows the determination of 3 mutually perpendicular eigenvectors, which coincide with the main water movement direction in axons in white matter. Combinations of these 3 vectors allow the derivations of 2 principal indices: overall mean water diffusion (MD) and the directionality or anisotropy of diffusion (FA). Reductions in white matter FA values have been identified with degradation of axonal integrity and a loss of cortical connections.[11] Diffusion increases in conditions leading to neuronal shrinkage or increased extracellular space.[12] Several authors, including the Candidate, have imaged human mild TBI subjects with DTI.[9, 13-17] Within the first 72 hours of mild TBI, FA is increased and MD is decreased suggesting axonal swelling.[9, 15] At later time points after mild TBI, the reverse pattern of DTI indices is seen with FA decreased and MD increased.[13, 14, 17] This has been speculated to represent axonal (or neuronal) loss. In the current proposal, DTI will be used to quantify AI occurring after mild TBI

pNFH 1 as Possible Marker of AI Neurofilaments (NF) are the most abundant protein structures in neurons. They are made up of 3 interlocking proteins, NF light chain, NF medium chain, and NF heavy chain. Unlike NFL and NFM, NFH undergoes extensive post-translational phosphorylation making it resistant to degradation by proteases. The phosphorylated form of NFH (pNFH) is found exclusively in neuronal axons.[18] These two features make pNFH an ideal potential marker of AI. Elevated levels of pNFH have been found in the CSF of patients with relapsing remitting multiple sclerosis and in the serum of humans with acute optic neuritis.[18] pNFH levels have not been reported after TBI in humans. However, after CCI in rats, pNFH rises slowly after injury, peaking at day 2 and returning to control levels by day 7. [19] A recent collaboration with Gerry Shaw, PhD, the developer of the Gainsville pNFH ELISA, allowed us to measure pNFH in the serum of moderate to severe TBI patients at our institution (see Preliminary Studies). The potential specificity of pNFH for AI, along with these promising preliminary data, have encouraged us to analyze this protein for its role in AI.

11B. Innovation

ApoA1 after Mild TBI We observed a rapid and robust increase in serum ApoA1 after mild TBI. (see Preliminary Studies) Exploring the role of this non-CNS protein in AI and recovery from AI represents a novel and innovative aspect of this proposal. ApoA1 is synthesized in the liver, is not found in brain, and is one of the principle components in high density lipoproteins (HDL) particles.[20] HDLs are anti-atherogenic although the exact mechanism of this function is not known. ApoA1 interacts with HDL as part of the reverse cholesterol transport pathway.[21] In this pathway, HDL particles transport cholesterol back to the liver for excretion or to other tissues that use cholesterol to produce hormones.[22] ApoA1 induction may provide a mechanism by which peroxidated or otherwise damaged lipids are removed from the CNS after TBI. Indirect evidence for this comes from a study revealing elevations of ApoA1 in the CSF of severe TBI patients. These ApoA1 particles were relatively small and contained only phospholipids and cholesterol. The authors speculated that the small ApoA1 particles are formed when rafts of phospholipid and free cholesterol are sheared from neuronal membranes after neuronal death.[23] These prior studies and our preliminary data form the basis for SA 2.

Global Epigenetic Changes After Mild TBI Exploring changes to DNA before and after mild TBI represents another innovative aspect of this proposal. There is a strong observed risk between TBI and the later development of dementia of the Alzheimer's type.[2] Epigenetic changes to DNA could be the mechanistic link between AI and neurodegeneration. These changes, such as DNA methylation and histone acetylation, chemically modify DNA such that gene transcription is blocked. Failure to repress genes, via demethylation or deacetylation, has been connected to several neurodevelopmental disorders.[24] Recently, Mastroeni and Coleman found epigenetic differences in the brains of monozygotic twins discordant for Alzheimer's Disease (AD) examined post mortem. Significantly decreased methylation in the neurons of temporal neocortex and superior frontal gyrus were found in the AD twin.[25] These same changes have been analyzed in the DNA of peripheral leukocytes.[26] Similar reductions in DNA methlylation and histone deacetylation have been found in the brains of rats after controlled cortical impact. These studies, and our own preliminary data, have led to our hypothesis that the link between TBI and dementia is related to these epigenetic changes, and that these changes may be detectable through an examination of DNA changes in peripheral leukocytes.

Insights into AI From Serum Protein Exposure Characteristics We propose to examine serial changes in serum and/or CSF proteins in subjects with mild TBI as well as in those with moderate to severe TBI. These serial changes will provide insight into the natural history of AI and its relationship to recovery in athletes. By obtaining serum samples pre-injury and at four time points after injury, fluctuations in protein levels with time can be directly observed and correlated to DTI-demonstrated AI and cognitive function. In moderate to severe TBI subjects, serum protein changes can be related to CSF protein changes and BBB permeability as well as fluctuations in clinical course. Longitudinal analysis of protein biomarkers expressed in specific cell types or sub-cellular compartments within the brain allows for a non-invasive method to gain insight into the mechanisms of AI. This method was recently employed in an analysis of 3 alpha II-spectrin breakdown proteins in the CSF of 30 human subjects with severe TBI.[27]

Use of Athlete Cohorts to Study Serum Protein Exposure Characteristics Proper interpretation of serum CNS protein concentrations requires comparison to an uninjured control group. Any comparison to unrelated controls will be confounded by significant individual variation in proteins levels and in cognitive function. A study design in which each subject serves as his own control would permit a direct statistical comparison of the changes in level of a putative protein using a relatively small sample size. The use of athletes participating in sports with a high risk of concussion permits investigators to obtain serum and cognitive performance before injury at the beginning of the season and then again after concussion. A recent collaboration between the Department of Emergency Medicine and the University of Rochester

Athletic Department allows the Candidate access to all undergraduate athletes, where potential subjects for this study can be recruited. The head athletic trainer for University of Rochester, Eric Rozen, is a co-investigator on the current project. (see Support Letter)

11C. Approach

Overview of research to be specifically supported by this grant In order to carry out the proposed Specific Aims, two subject cohorts will be assembled. (**Table 8**) In the first cohort, 160 college athletes from the University of Rochester will be enrolled pre-sports participation over approximately 18 months. These subjects will be followed for the duration of their time at the University (4 years). Forty subjects who sustain a sport-related concussion during this time will undergo subsequent serum sampling, DNA testing, cognitive testing, and DTI. The participants in this study will serve as their own controls. In the second cohort, 15 moderate to severe TBI patients who require the insertion of an external ventriculostomy drain for clinical care will be enrolled over approximately four years. Paired serum and CSF samples will be obtained at several time points after their injury.

Table 8 Overview of Proposed Study Activities

Cohort	Activity	Time After Injury					Specific Aim
		Pre-injury	24 hrs	2 days	3 days	7 days	
Mild TBI Athletes n=40	Serum	ApoA1 pNFH	ApoA1 pNFH	ApoA1 pNFH	ApoA1 pNFH	ApoA1 pNFH	1 & 2
	Imaging			DTI			1
	Cognitive Testing	ImPACT			ImPACT	ImPACT	2
	Whole Blood	DNA				DNA	3
Mod-Sev TBI n=15	Serum		ApoA1 pNFH, alb	ApoA1 pNFH, alb	ApoA1 pNFH, alb		1 & 2
	CSF		ApoA1 pNFH, alb	ApoA1 pNFH, alb	ApoA1 pNFH, alb		1 & 2

Research Methods Related to Specific Aim 1

Athlete cohort

Inclusion and Exclusion Criteria: Subjects will be recruited from University of Rochester athletes who participate in varsity football, soccer, and basketball. Subjects will be eligible for participation if they are ≥ 18 years of age. Subjects who sustain a moderate or severe TBI (GCS of 3-12), or who are pregnant at the time of injury (MRI risk) will be excluded. Consenting subjects will have baseline blood and cognitive testing done at the beginning of the sports season. ***Determination of Mild TBI:*** Subjects will be considered to have suffered a mild TBI if they are suspected by the on-field trainer of having had a head injury and they score <25 on the Standardized Assessment of Concussion (SAC).The SAC is a brief screening instrument that assesses orientation, immediate memory, concentration, and delayed recall, summing to a total composite score of 30 points.[28] It is anticipated that approximately 12-15 athletes each year will suffer a concussion. This estimate comes from the Head Athletic Trainer, Eric Rozen (see Support Letter). ***Limitations:*** Subject accrual rates are difficult to guarantee and could fall short of projections. The Candidate is in the process of expanding this pilot to athletes at the Rochester Institute of Technology. This institution has athletes active in a variety of contact sports. Including this institution has potential to double the number of available subjects. (See Support Letter from Athletic Director, Ben Emke) ***Post-injury Activities:*** Participants who suffer a mild TBI during the course of the season will have blood and cognitive testing repeated at four time points after injury, per Table 8. ***Serum Collection and Storage***: At each study interval, 15cc of blood will be obtained. After appropriate preparation, samples will be stored at -80C until analysis. ***Limitations:*** Prolonged storage of serum samples could lead to protein degradation if mild TBI results in activation of proteases. Because serum from each blood draw will be

aliquoted into several tubes, we will be able to compare the concentrations of pNFH and ApoA1 assayed at the time of injury to concentrations of these proteins assayed after 12 months of storage. This will allow us to estimate the effect, if any, of prolonged storage on protein integrity.

Moderate to Severe TBI cohort

Inclusion and Exclusion Criteria: Subjects are eligible for inclusion if they are ≥ 16 years old, have a history or physical exam evidence of blunt, non-penetrating head trauma, and have a ventriculostomy catheter placed as part of clinical care within 12 hours of injury. ***CSF and Serum Collection and Storage:*** Blood and CSF will be drawn at the 3 time intervals indicated in Table 8. After appropriate preparation including centrifugation, all samples will be stored at –80C until analysis. ***pNFH analysis in Serum and CSF*** Serum and CSF concentrations of pNFH will be determined using the Gainesville ELISA. (see Dr. Shaw Support Letter) ***Determining BBB Permeability using Albumin Quotient*** The current gold standard technique for determination of BBB permeability is via the albumin quotient (Q_A), defined as Q_A=[albumin$_{CSF}$]/[albumin$_{serum.}$]. Determining BBB permeability using this method requires the simultaneous measurement of serum and CSF albumin (alb). Normal BBB permeability is defined as a $Q_A \leq 0.007$.[29]

Diffusion Tensor Imaging DTI scanning will take place at Rochester Center for Brain Imaging under the supervision of Dr. Jianhui Zhong (see Support Letter). Images will be acquired with a 3T Siemens Trio scanner. DTI will be performed using a single-shot pulsed-gradient SE-EPI sequence. Acquisition parameters are: TR/TE= 4800/91 ms, slice thickness=3 mm with no gap, matrix=128x128, FOV=25.6cm, iPAT (GRAPPA) acceleration factor =2, DWI direction=6 with 4 averages, b=1000. From the DTI images, FA, overall MD, radial MD and axial MD will be analyzed. Probabilistic tracking methods will be used to identify areas in white matter with multiple fiber crossing. These areas will be excluded to minimize possible false alteration of DTI measures. ROIs will be manually outlined on T1 images and projected to the co-registered DTI images. A comprehensive registration routine from home-built software will be used to minimize spatial misregistration resulting from the more severe distortions in EPI-based diffusion tensor images due to susceptibility and eddy-current artifacts. These steps are described elsewhere in full detail.[9]

Research Methods Related to SA 2

ApoA1 analysis in serum and CSF Mabtech's ELISA (ELISA^{PRO}) will be used to quantify ApoA1 concentration in CSF and plasma.

Cognitive testing Cognitive testing will be accomplished using ImPACT, a proprietary computer program that measures verbal memory, visual memory, reaction time, and visuomotor speed. ImPACT also includes a symptom inventory. In order to minimize the practice effect the questions are randomized each time the program is run.[30]

Research Methods Related to SA 3

After centrifugation, the buffy coat will be collected and used for isolation of genomic DNA and immunocytochemical analysis of methylation and histone acetylation antibodies. Immunocytochemical analysis will involve several preparation steps, the application of the appropriate primary antibodies, followed by application of biotinlyated secondary antibody. For DNA extraction, QIAamp DNA mini and Blood Mini Kits will be used following the protocols provided with the kits. These steps are describe elsewhere in full detail.[25]

Analysis

SA1: Using results from the athlete cohort, changes in pNFH level on Day 2 will be correlated to mean FA in whole brain and in 5 regions of interest from DTI performed on Day 2 using

Spearman's correlation coefficient. FA measures will be derived using a quantile approach, which is described in the candidate's prior DTI publication in full detail.[9] SA 1: Using results from the moderate-severe TBI cohort, the albumin quotient (Q_A) will be calculated in each subject at each post injury time point. The ratio of pNFH in serum to pNFH in CSF will be correlated to the Q_A among all subjects at each post-injury time point using a Spearman's correlation coefficient. SA 2: Using results from the athlete cohort, changes in ApoA1 protein levels at Day 3 and 7 will be correlated to changes in 4 indices of cognitive function from the ImPACT test (verbal memory, visual memory, reaction time, and visuomotor speed) performed on those same days using Spearman's correlation coefficient. SA 3: The mean fluorescence intensities for the 5-methylcytosine indicator in leukocyte nuclei will be compared in athletes after injury to those before injury using students t-test.[25]

Sample Size and Power Athlete Cohort: SA 1 will be used to determine the sample size required to detect a significant correlation between pNFH levels and changes in FA on DTI. Shown in the table below are the sample sizes needed to detect a range of correlations (r) that are significantly different from there being no correlation (i.e. that the correlation would be 0) given a Type II error rate of 20% (ie, power of 80%) and 2 levels of Type I error, 0.05 and 0.10.

Expected r	Sample Size (α=0.05)	Sample size (α =0.10)
0.3	84	67
0.4	46	37
0.5	29	23
0.6	19	16
0.7	13	11

Based on our accrual rate of 5 concussed athletes in the first 5 months of the pilot period, and assuming athlete participation in contact sports 10 months out of the year, we anticipate accruing 40 subjects by the end of the 4 year study period. This sample size would allow us to detect correlation coefficients as low a 0.4. Because accrual rates are difficult to guarantee, we are in the process of expanding our pilot efforts to athletes at the Rochester Institute of Technology. This institution has athletes active in a variety of contact sports. Including this institution as a study site will potentially double the number of available subjects. (See Support Letter from Athletic Director, Ben Emke) Mod-severe TBI Cohort: SA 1 will be also be used to determine the number of subjects needed to estimate the relationship between BBB permeability (Q_A) and the $[pNFH]_{serum}$/ $[pNFH]_{CSF}$ ratio. To estimate a correlation r to within +/- 0.4 with 95% confidence requires n=19 independent subjects (fewer if r is far from 0), while n=13 subjects provide 90% confidence with the same precision. Fifteen subjects thus will provide between 90 and 95% confidence for this correlation estimate.

Preliminary Studies For New Applications

DTI after Mild TBI DTI images of 13 mild TBI patients were compared to 21 age- and gender-matched orthopedic controls in 48 white matter structures at 24 hours, 1 week, and 1 month after injury. We observed decreased FA values in mild TBI subjects compared to controls at all three time points. At 24 hours, decreases in FA were statistically significant ($p<0.05$) in 4 white matter structures (**Table 8**, bolded p-values) and approached significance ($p<0.2$) in two white matter structures. There were fewer differences in mean diffusivity (MD), axial diffusivity and radial diffusivity (not shown). Significant decreases in FA were spatially mapped in **Figure 1**B.

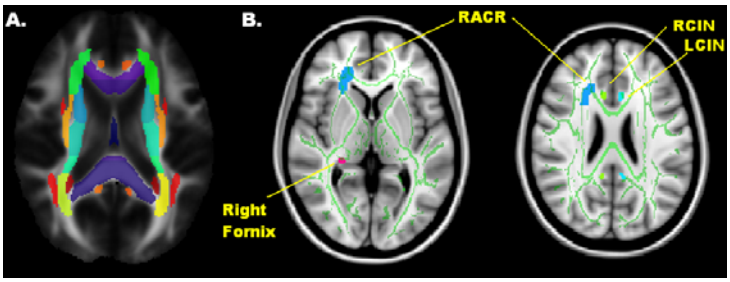


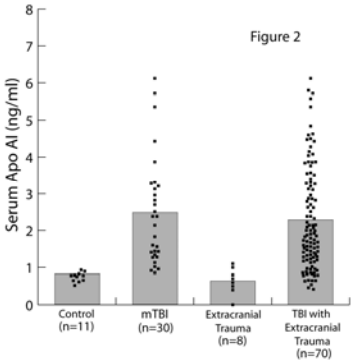
Figure 1 DTI Mild TBI vs Controls at 24 hours (A) The John Hopkins human brain white matter atlas is superimposed onto the mean FA maps of all subjects. **(B).** Regions with significantly decreased FA (blue)are superimposed on the standard T1 brain templates. The mean FA skeleton (green) is used to correlate the fiber tracts with the white matter atlas. Anterior Corona Radiata (ACR), Cingulum (CIN).

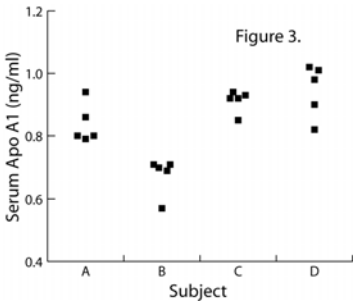
White matter structures	FA			MD		
	mTBI	Cont	p	mTBI	Cont	p
ACR (Right)	0.409	0.430	**0.043**	0.864	0.862	0.869
CIN (Right)	0.430	0.449	**0.026**	0.803	0.806	0.777
CIN (Left)	0.463	0.482	**0.030**	0.808	0.804	0.697
Fornix (Right)	0.432	0.453	**0.035**	1.007	1.010	0.881
MCP	0.430	0.440	0.061	0.713	0.731	0.113
ILF + IFOF (Left)	0.463	0.474	0.199	0.943	0.985	**0.046**

Table 8 DTI Mild TBI vs Controls at 24 hours: Anterior Corona Radiata (ACR), inferior fronto-occipital fasciculus (IFOF), Inferior longitudinal faciculus (ILF), Middle cerebellar peduncle (MCP), Cingulum (CIN).

These results demonstrate the Candidate's ability to detect early AI in mild TBI subjects using changes in FA on DTI. These same methods will be employed in SA 1.

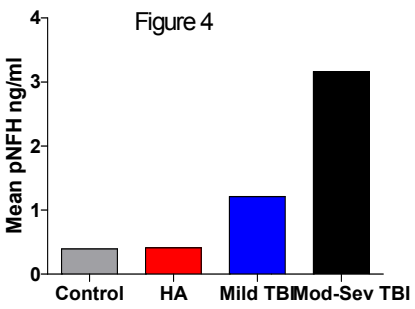
Proteomic Discovery of ApoA1 The serum proteome of mild TBI subjects with high 3-month post-concussive symptom (PCS) scores was compared to those with low PCS scores using banked serum samples and 2-D gel electrophoresis. Significant differences between the two groups were found in the intensity of 20 gel spots ($p<0.05$), which were removed and subjected to protein identification via mass spectroscopy. From this, 2 intact proteins were identified; serpin and ApoA1. The levels of these 2 proteins were determined in an independent group of banked serum samples using commercially available ELISA kits (Mabtech). Although serpin did not show a statistically significant relationship with TBI severity or outcome, ApoA1 did. There was a statistically significant increase in ApoA1 levels among isolated mild TBI subjects compared to uninjured controls ($p<0.001$) and controls with extracranial trauma ($p<0.001$), as well as among mild TBI subjects with extracranial trauma compared to uninjured controls ($p<0.001$) and controls with extracranial trauma ($p<0.001$). **Figure 2** This suggests that ApoA1 is released specifically in response to TBI and is not simply a non-specific response to injury. These results have encouraged us to further study this protein and its possible role in AI.

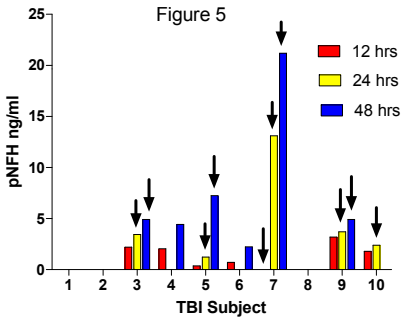




Limitations: Because banked serum samples used to evaluate ApoA1 may have been subjected to various freeze-thaw cycles, we examined serum ApoA1 levels among 4 uninjured controls whose samples were subjected to 5 different freeze-thaw cycles (0, 1, 2, 3 and 4 cycles). **Figure 3** All samples were run in quadruplicate. There were no significant differences in mean ApoA1 levels for the different freeze-thaw cycles. These results suggest that various freeze-thaw cycles do not appear to affect ApoA1 levels, and support our proposal to collect serum samples over a 4 year time period.

Longitudinal changes in pNFH after TBI suggest role in AI

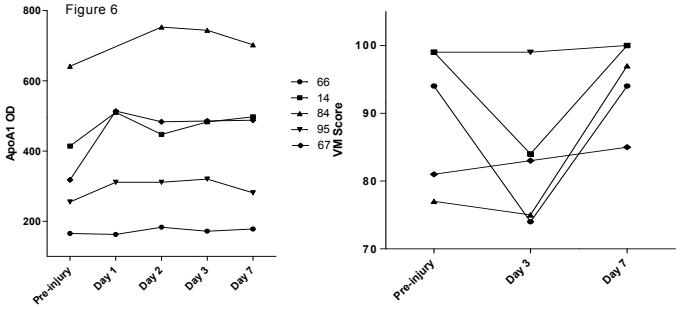




We measured serum pNFH in 10 patients with moderate-severe TBI 12 hours, 24 hours, and 48 hours after injury using Gainsville ELISA. Levels were compared to 4 healthy controls, 3 migraine headache subjects and 4 mild TBI subjects. Combining results from all time points, (**Figure 4**) mean

serum pNFH levels were considerably higher in moderate to severe TBI subjects (3.16 ng/ml) compared to controls (0.395 ng/ml, p=0.27), headache subjects (0.41 ng/ml, p=0.34), and mild TBI subjects (1.21 ng/ml, p=0.43). None of these comparison reached statistical significance likely because of the small number of subjects. Note the appearance of pNFH in serum when albumin quotient is <0.007, ie, BBB is closed (**Figure 5**, arrowheads). pNFH's reported specificity for AI and its delayed rise suggests that it is released early in response to AI but only gradually diffuses into the serum. Correlation with the albumin quotient suggests that diffusion of pNHF from CSF into serum does not require an open BBB. We propose to test both of these hypotheses.

ApoA1 and Cognitive Testing in Athletes Demonstrate Feasibility of Study Design In the first 4 months of piloting the athlete cohort, 5 subjects suffered a concussion. Serum was analyzed for ApoA1 via Western blotting and shown in L panel of **Figure 6**. In 4 of the 5 subjects, ApoA1 levels (relative optical density) increased immediately after injury, decreasing to pre-injury levels by day 7 in 3 subjects. Visual memory scores (R panel) decreased immediately after injury in 3 of 5 subjects, returning or exceeding pre-injury performance in all subjects by day 7. A greater number of subjects will permit a statistically meaningful correlation of changes in ApoA1 levels to changes in cognitive performance. We propose to use this before and after study design with athletes in the current proposal.



Epigenetic Changes in Mice Brain after TBI Suggests Link to Neurodegeneration Mice where injured in the Candidate's lab by CCI and sacrificed at 4 hours, 24 hours or 7 days after injury. Brains were stained for DNA methylation (**Figure 7a**) and histone deacetylase 2 expression (**Figure 7b**) in Dr. Coleman's lab (see Support Letter).These initial images show substantial decreases in staining for both DNA methylation and histone deacetylation as a consequence of experimental TBI. Since similar changes have been found in Alzheimer's patients, our results suggest a link between mild TBI and neurodegeneration. We propose to examine these changes in leukocytic DNA before and 7 days after mild TBI in the athlete cohort.

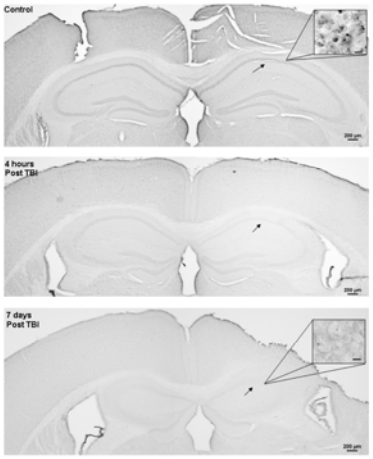


Figure 7A. DNA methylation in mouse brain at 4 hours (middle panel) and 7 days (bottom panel) after experimental TBI by controlled cortical impact.

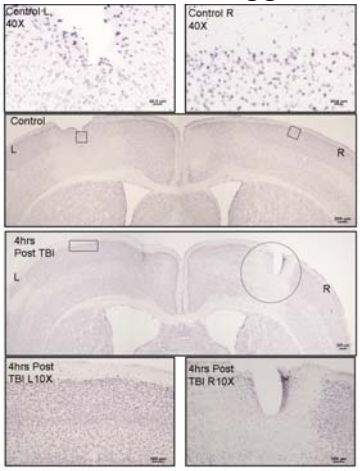


Figure 7B. Histone deacetylase 2 staining in mouse brain 4 hours after experimental TBI by controlled cortical impact.

1. RISKS TO SUBJECTS

a. Human Subjects Involvement and Characteristics:

Number of subjects: Athlete cohort-160 initially enrolled, 40 expected to develop mild TBI over four years. Moderate to severe TBI cohort-15 subjects over 4 years.
Gender of subjects: Both males and females are eligible for inclusion.
Age of subjects: Athlete cohort ≥18 years. Moderate to severe TBI cohort ≥16 years.
Racial and Ethnic Origin: All races and ethnicities are eligible. In the athlete cohort, ability to speak and read English or Spanish is necessary because the computerized cognitive outcome outcome tool (ImPACT) is available only in English and Spanish.
Vulnerable Subjects: Students are eligible for inclusion. Pregnant females are not eligible for inclusion in the athlete cohort due to the potentially deleterious effects of magnetic field in DTI. Prisoners and wards of the state are unable to return for follow-up testing and thus are not eligible.

b. Sources of Materials: Information collected directly from subjects.

c. Potential Risks:

In the athlete cohort, the subject's name and contact information will be maintained in a computer database for 4 years. This is necessary in order to link post injury information with pre-injury data. Subjects will incur no costs. Costs for the blood draws, assays, cognitive testing and DTI will be covered by the investigators. Serum testing: The risks associated with drawing blood include pain, bleeding and bruising at the site where the blood was taken. There is also the risk of becoming lightheaded or even to faint. There is always the slight risk of skin infection. CSF removal from ventriculostomy catheter: The main risk is of inadvertent entry of infection into the catheter and the brain ventricle. Cognitive testing: Cognitive testing is a physically safe procedure, but there is potential for psychological risks to the subjects. There is potential for an adverse adjustment in self-image based on poor performance in the tasks of the neurobehavioral test. In addition the patient could perceive stress associated with the time pressure of some the cognitive tasks. DTI: Magnetic resonance imaging (MRI) has been used clinically for more than 20 years. It has been proven safe without any lasting adverse reactions at this field strength, 3T, when care is taken to screen patients for MR-contraindications before entering the MR site, e.g. pace-maker, shrapnel, etc. All the studies will be performed using the new research MR scanner employing standard or equivalent RF pulse sequences and hardware that have been approved by the FDA for human clinical use. The field strength is 3 Tesla and all other relevant operating characteristics (RF power deposition, rate of change of the field gradients, coil design) fall within the limits of the FDA guidelines for MRI exposures. Claustrophobic reaction during scanning is a known psychological risk of MRI. The only viable alternative to participation in the study is refusal to participate at the time of consent, or withdrawal from participation at any time during the course of the study. Both options are open to all subjects and are presented to all subjects during the consent procedure.

2. ADEQUACY OF PROTECTION AGAINST RISKS

a. Recruitment and Informed Consent:

Athlete Cohort: Potential patients will be recruited by the Head Athletic Trainer, Eric Rozen (see Support Letter). Study coordinator will screen eligible patients using the aforementioned inclusion and exclusion criteria to determine eligibility. If still eligible, patients will be asked to sign informed consent. The consent form will provide the subject with a brief overview of the study, the purpose for colleting data and blood, and for performing cognitive testing and DTI. The consent form will describe these procedures, as well as the risk and benefits of

participation, the voluntary nature of participation, how information will be kept confidential, and research contact persons. A copy of the consent form will be given to the subject, and the original will be kept on permanent record with the rest of the patient's enrollment materials. The Research Subject Review Board of the University of Rochester will approve the proposal and consent form before enrollment begins. All subjects will have the capacity to give informed consent. Subject comprehension of all the elements of informed consent will be determined by the study coordinator who has been trained in the responsible conduct of human research. ***Moderate to severe TBI cohort:*** The neurosurgeon inserting the ventriculostomy catheter in a TBI patient will review the inclusion and exclusion criteria. (see Support Letter from Neurosurgery Department Chair, Dr. Pilcher) If the subject meets the study criteria, the neurosurgeon will identify an adult family member or, if the subject is <18 years old, a parent/ guardian, to sign informed consent within 24 hours. Per URMC policy regarding research on decisionally impaired subjects, family members will be approached in the following order to provide research consent: spouse, adult son/daughter, parent/guardian, adult sibling, friend with close affinity. Because TBI patients often present without an adult family member or parent/guardian to provide consent, a portion of the additional leftover serum and CSF drawn for clinical reasons from the subject in the first 24 hours will be collected and stored. These samples will not be analyzed until signed consent is obtained. Additional blood/CSF samples and data will not be collected unless and until consent is signed. If informed consent cannot be obtained within 24 hours of the patient's arrival to the hospital, the saved serum and CSF samples will be discarded.

b. Protection Against Risk:

Serum testing: Study personnel drawing blood are trained in sterile technique. This will help to minimize the pain and discomfort associated with a needle stick. Cognitive testing: Proper instruction and supervision of the test can minimize the psychological risks associated with neurobehavioral testing. This will be done by one of the investigators trained in the use and administration ImPACT. CSF removal from ventriculostomy catheter: This will only be performed by neurosurgeons, who are trained in ventriculostomy catheter placement and CSF removal. It is standard practice in the neurosurgical ICU to send for microbiological analysis a small sample of CSF every 48 hours to monitor for occult infection. DTI: To minimize the injury due to the exposure to the high magnetic field during the MRI scanning all participants will undergo rigorous screening procedure using standardized screening procedures used for regular clinical MR examinations, which includes participant's history of brain and head surgery, heart and chest surgery, ear and eye surgery, and history of bullets, shrapnel, BB, pierced body parts, etc. This screening will be performed at the time of enrollment into the study, and will be confirmed at the time the DTI is performed. This screening procedure will also ask about the patient's perceived risk of becoming claustrophobic while in the MRI scanner. Patients who say they might become claustrophobic will not be eligible for inclusion. Patients who become claustrophobic while scanning will be immediately removed from the scanner and withdraw from the study. Women of childbearing potential will have a urinary pregnancy test done prior to scanning. Pregnant women will be excluded.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

Subjects will receive no benefit from participation in this study. In the athlete cohort, subjects will receive the cognitive testing and the results free of charge. Clinically significant deficits detected will be referred to Eric Rozen, the head Athletic Trainer, for further evaluation and treatment. The results of serum testing or DTI will not be communicated to the subjects as the clinical

significance of abnormalities in these modalities is not known. In the moderate to severe TBI cohort, results of serum and CSF testing will not be communicated to the subjects as the clinical significance of abnormalities is not known. Future mild TBI patients will benefit tremendously from the results of this study. Early detection of brain injury with a DTI or a blood test could prevent future patients from receiving unnecessary brain CT scans with the attendant risks of radiation exposure and pre-procedural sedation.

4. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

Mild TBI is an important public health problem in the US for which there is currently no objective diagnostic aid and no treatment. Not only does this injury affect 1.2 million Americans annually, it also frequently affects US soldiers involved in combat operations abroad and public safety personnel who survive terrorist attacks. Disturbances in simple reaction time and other cognitive functions can interfere with the important duties these individuals must perform, putting themselves and others at risk. A better understanding of AI through an evaluation of changes in CSF and blood proteins could lead to the development of future therapies.

Considering both subject cohorts, it is anticipated at 40% of eligible subjects will be women. (See Targeted/Planned Enrollment Table) Every attempt will be made to ensure that women are not inadvertently excluded from enrollment. There will be no specific attempt to recruit women as research subjects. Because MRI poses a theoretical risk to the fetus, pregnant women are not eligible for inclusion in the athlete cohort. Based on the racial and ethnic makeup of patients who participated on University of Rochester varsity sports during 2008 and in subjects in the moderate to severe TBI pilot study, it is anticipated at 10% of eligible subjects will be Hispanic or Latino, 30% will be Black or African American, and 5% will be Asian. (See Targeted/Planned Enrollment Table) Every attempt will be made to ensure that minorities are not inadvertently excluded from study. There will be no specific attempt to recruit minorities as research subjects. In the athlete cohort, patients unable to speak and read English or Spanish will be excluded because the computerized cognitive test instrument is available only in English and Spanish. At this time all University of Rochester athletes can speak and read English. In the recording of race and ethnicity data in the current proposal, the categories established by the Office of Management and Budget Standards (OMB Directive No. 15) will be used.