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GENETICS IN EXPERIMENTAL TRAUMATIC BRAIN INJURY

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Cover Image: The Crescent “Brain” nebula in the constellation Cygnus and the Double Helix “DNA” nebula near the centre of our galaxy.

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ABSTRACT

Traumatic brain injury (TBI) is the leading cause of death and disability in the young population in the industrialized world. It comprises a heterogeneous group of brain pathologies where head trauma initiates a series of complex molecular pathways, which, together with the initial injury, account for the final outcome. Although extensive research has shed some light on these pathways, they are still incompletely understood. No pharmacological treatment for TBI exists. This project was initiated to study a possible impact of genetic heterogeneity in experimental TBI and identify genes/loci that regulate the secondary TBI pathways and outcome.

Brain contusion was induced using the weight drop injury (WDI) model in inbred and congenic rat strains. Inflammatory pathways, infiltration of neutrophils, NK cells and monocytes/macrophages and activation of microglia and the complement pathway were found to be regulated by *non-MHC* (Major Histocompatibility Complex) genes. Non-MHC genes did also influence neurodegeneration, and interestingly, a stronger inflammatory response was correlated to a more vigorous neuronal/axonal injury and neurodegenerative outcome. Further, the use of congenic rats with loci harboring the *Ciita* gene or the *MHC*-gene complex, revealed that mainly *MHC* genes regulate MHC-II presentation after TBI with a smaller contribution from *Ciita*, and also that *MHC* genes regulate a delayed T cell infiltration after TBI, suggesting a role for adaptive immune responses and autoimmunity in TBI.

We used various genetic mapping approaches to disclose genes that regulate neurodegeneration in a rat ventral root avulsion (VRA) model and found that glutathione-S-transferase alpha 4 (*Gsta4*) is a candidate gene for regulating motorneuron death in this model. Levels of *Gsta4* were genetically regulated by a variation in the *Gsta4* gene region and had an inverse correlation to the degree of neurodegeneration. This effect of *Gsta4* gene variation was replicated in experimental TBI where it regulated the degree of hippocampal neuronal cell loss. *Gsta4* exerts its effect possibly via more efficient detoxification of the highly reactant product of lipid peroxidation, 4-hydroxynonenal (4-HNE). The presence of 4-HNE was demonstrated in experimental TBI and also in human pericontusional tissue providing evidence for the importance of the *Gsta4* – 4HNE pathway also in human TBI. Taken together, the findings in both VRA and TBI suggest that the *Gsta4* - 4-HNE detoxification pathway can be important, not only in TBI, but possibly also in other neurodegenerative diseases.

Alltogether, the findings of this thesis demonstrate that genetic heterogeneity has a substantial impact on the secondary pathways and outcome in experimental TBI and highlight the need for further research in the field of genetics in TBI.

LIST OF PUBLICATIONS

This thesis is based on the following studies, which will be referred to in the text by their Roman numerals:

- I. Al Nimer E, Lindblom R, Ström M, Guerreiro-Cacais AO, Parsa R, Aeinehband S, Mathiesen T, Lidman O, Piehl F.
Strain influences on inflammatory pathway activation, cell infiltration and complement cascade after traumatic brain injury in the rat.
Brain Behavior and Immunity; In Press. PMID: 23044177

- II. Al Nimer E, Beyeen AD, Lindblom R, Ström M, Lidman O, Piehl F.
Both MHC and non-MHC genes regulate inflammation and T-cell response after traumatic brain injury.
Brain Behavior and Immunity, 2011; 25(5):981-90. PMID: 20974248

- III. Ström M, Al Nimer F*, Lindblom R* , Nyengaard JR, Piehl F.
Naturally Occurring Genetic Variability in Expression of Gsta4 is Associated with Differential Survival of Axotomized Rat Motoneurons.
Neuromolecular Medicine, 2012; 14(1):15-29. PMID: 22160604

- IV. Al Nimer F*, Ström M*, Lindblom R, Bellander BM, Nyengaard JR, Lidman O, Piehl F.
Naturally occurring variation in the glutathione-S-transferase 4 gene determines neurodegeneration after traumatic brain injury.
Antioxidants & Redox Signaling; In Press. PMID: 22881716

* These authors contributed equally to the work

ADDITIONAL PUBLICATIONS

Related publications not included in the thesis:

Al Nimer F, Wennersten A, Holmin S, Meijer X, Wahlberg L, Mathiesen T.

MHC expression after human neural stem cell transplantation to brain contused rats.

Neuroreport, 2004; 26; 15(12):1871-5.

Rudehill S, Muhallab S, Wennersten A, von Gertten C, Al Nimer F, Sandberg-Nordqvist AC, Holmin S, Mathiesen T.

Autoreactive antibodies against neurons and basal lamina found in serum following experimental brain contusion in rats.

Acta Neurochirurgica (Wien), 2006; 148(2):199-205

Wennersten A, Holmin S, Al Nimer F, Meijer X, Wahlberg LU, Mathiesen T.

Sustained survival of xenografted human neural stem/progenitor cells in experimental brain trauma despite discontinuation of immunosuppression.

Experimental Neurology, 2006; 199(2):339-47.

Diez M, Abdelmagid N, Harnesk K, Ström M, Lidman O, Swanberg M, Lindblom R, Al-Nimer F, Jagodic M, Olsson T, Piehl F.

Identification of gene regions regulating inflammatory microglial response in the rat CNS after nerve injury.

Journal of Neuroimmunology 2009; 25;212(1-2):82-92.

Günther M, Al Nimer F, Gahm C, Piehl F, Mathiesen T.

iNOS-mediated secondary inflammatory response differs between rat strains following experimental brain contusion.

Acta Neurochirurgica (Wien), 2012; 154(4):689-97

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LIST OF ABBREVIATIONS

2D	Two-Dimensional
3D	Three-Dimensional
4-HNE	4-Hydroxynonenal
8-epi PGF _{2a}	8-epi Prostaglandin F _{2a}
APC	Antigen Presenting Cell
ACE	Angiotensin-Converting Enzyme
AGER	Advanced Glycation Endproduct Receptor
AIF1	Allograft Inflammatory Factor 1
APOE	Apolipoprotein E
BBB	Blood Brain Barrier
BDNF	Brain-Derived Neurotrophic Factor
C1, 2, 3 or 4	Complement Component 1, 2, 3 or 4
CACNA1A	Calcium Channel, Voltage-dependent, P/Q type, Alpha 1A Subunit
CCI	Controlled Cortical Impact
cDNA	Complementary Deoxyribonucleic Acid
cFPI	Central Fluid Percussion Injury
Ciita	Class II Transactivator
CLIP	Class II-associated Invariant Chain Peptide
CNS	Central Nervous System
COMT	Catechol-O-methyltransferase
CSF	Cerebrospinal Fluid
CXCL1	Chemokine (C-X-C motif) ligand 1
DA	Dark Agouti
DAI	Diffuse Axonal Injury
DRD2	Dopamine Receptor D2
ELISA	Enzyme-Linked Immunosorbent Assay
EAE	Allergic Autoimmune Encephalomyelitis
GAPDH	Glyceraldehyde-3- Phosphate Dehydrogenase
GFAP	Glial Fibrillary Acidic Protein
GOS	Glasgow Outcome Scale
GPX	Glutathione Peroxidase
GR	Glutathione Reductase
GSH	Reduced form of Glutathione
GSSH	Oxidized form of Glutathione
GST	Glutathione S Transferase
HLA	Human Leukocyte Antigen

HPLC	High-Performance Liquid Chromatography
HPRT	Hypoxanthine Guanine Phosphoribosyltransferase
ICAM-1	Intercellular Adhesion Molecule 1
ii	Invariant Chain
IL	Interleukin
IFPI	Lateral Fluid Percussion Injury
LCN2	Lipocalin-2
LTA	Lymphotoxin Alpha
MAC	Membrane Attack Complex
MBP	Myelin Basic Protein
MDA	Malondialdehyde
MHC	Major Histocompatibility Complex
mRNA	Messenger Ribonucleic Acid
MS	Multiple Sclerosis
MSD	Meso Scale Discovery
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NEFH	Neurofilament Heavy
NFH	Neurofilament Heavy
NFL	Neurofilament Light
NFM	Neurofilament Medium
NK	Natural Killer
NOS	Nitric Oxide Synthase
NRF2	Nuclear Factor (Erythroid-derived 2)-like 2
P53	Tumor Protein 53
PARP-1	Poly(ADP-ribose) Polymerase-1
PNS	Peripheral Nervous System
PUFA	Polyunsaturated Fatty Acid
PVG	Piebald Virol Glaxo
qPCR	Quantitative Polymerase Chain Reaction
QTL	Quantitative Trait Locus
RLIP-76	Ral-interacting protein-76
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SCI	Spinal Cord Injury
SNP	Single-nucleotide Polymorphism
SOD	Super Oxide Dismutase
TBI	Traumatic Brain Injury
TGF-b	Transforming Growth Factor beta
TNF	Tumour Necrosis Factor
VRA	Ventral Root Avulsion
WDI	Weight Drop Injury

1 INTRODUCTION

Traumatic brain injury (TBI) is one of the most prevalent causes of morbidity and mortality around the world and the leading cause of death and disability in young adults in the industrialized world. The overall incidence of TBI in Europe ranges between 100 and 300 per 100,000 inhabitants depending on country and study. This causes an enormous social and economical burden, both for the individual and the society, and underscores the necessity to develop effective treatment strategies (Gustavsson et al. 2011). In the last decades, many experimental and clinical studies, aimed to test and find pharmacological treatments for TBI, have been performed. Even if some of the pharmacological agents work in the experimental setting, all studies have failed to introduce an effective treatment for human TBI.

Traumatic brain injury comprises a heterogeneous group and can include a wide range of vascular and/or parenchymal pathologies. Vascular injuries are classified as epidural, subdural, intracerebral and subarachnoidal hemorrhage, whereas parenchymal injuries are classified as focal contusions, lacerations and diffuse axonal injury. Motor vehicle accidents, falls, sports and violence are the main causes of TBI and lead to the primary injury, which is characterized by an acute mechanical brain tissue disruption. The severity and type of the primary injury determines the degree of acute cell death and is followed by a secondary phase with the activation of several pathophysiological pathways that can limit or aggravate the injury; hence both phases determine the outcome. The secondary phase starts within minutes after the injury and can continue for years after it, thereby providing a time window where therapeutic strategies can be introduced.

The secondary injury cascades following traumatic brain injury, are complex and involve BBB breakdown, excitotoxicity, mitochondrial dysfunction as well as activation of inflammatory-, oxidative- and neurodegenerative pathways (Fig. 1). Moreover, it is becoming increasingly clear that the individual's genetic predisposition can modify the response along these pathways. In this introduction, the secondary cascades that are relevant in the context of this thesis are reviewed, and data on the activation and role of these cascades in TBI are provided. Also, the current evidence on the role of genetics in TBI is reviewed.

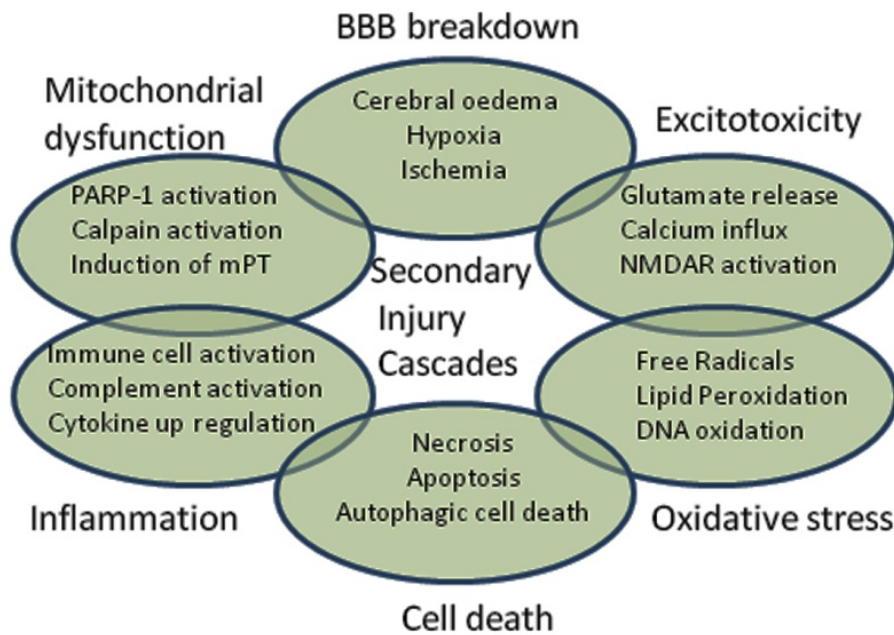


Figure 1. Secondary injury cascades following TBI.

1.1 INFLAMMATORY RESPONSE

Historically, it was believed that the central nervous system (CNS) is protected from the immune system and has a limited possibility to evoke an inflammatory response because of its protection by the intact blood brain barrier (BBB). However, over the last decades, a rapidly growing body of evidence has demonstrated a wide range of bi-directional interactions between the CNS and the immune system and thereby established the field of neuroimmunology (or neuroinflammation). With regards to TBI, the neuroinflammatory response is facilitated by the fact that the primary impact causes rapid disruption of the BBB and acute brain cell death. By now, several aspects of the inflammatory response in TBI have been investigated. The studies that are relevant for the investigations included in this thesis are summarized below.

1.1.1 Immune Cells

Traumatic brain injury results in a rapid destruction of the BBB allowing blood cells and serum components to enter the injured tissue. Neutrophils are the first cell type to enter the brain parenchyma and their migration is facilitated also by chemotactic factors such as CXCL1 and IL-8 and up regulation of adhesion molecules on the endothelium such as ICAM-1 (Bell et al. 1996; Carlos et al. 1997; Clark et al. 1996; Holmin et al. 1995; Szmydynger-Chodobska et al. 2009; Whalen et al. 1999). Neutrophils can release multiple proinflammatory molecules and depletion of these

cells reduces oedema formation and tissue loss after TBI (Kenne et al. 2012). Neutrophil infiltration is followed by monocytes/ macrophages that enter the injured tissue already at 24 hours but peak at 6-7 days after TBI as shown by immunohistological investigations (Holmin et al. 1995; Soares et al. 1995). However, it has to be noted that studies on these cells in TBI are difficult with immunohistology because cell markers such as ED-1 and OX-42 stain both monocyte- and microglia-derived phagocytic cells. Monocytes/ macrophages can release immune mediators and phagocytose injured cells and debris. Lymphocytes and NK-cells have also been described in head trauma with some evidence about the role of B and T lymphocytes; albeit detailed knowledge about their function is still lacking (Clausen et al. 2007; Holmin et al. 1995; Holmin et al. 1998).

In addition to the immune cells invading the brain from the periphery, host tissue immune cells are also activated after injury. Microglial cells are the primary immune cell mediators of the CNS response to injury, infection and disease. Microglia were first described by del Rio-Hortega in the early part of the 20th century and account for 10 – 20% of the total cell population in the adult CNS. The origin of these cells has long been debated. Recent evidence shows that they belong to the mononuclear phagocyte lineage and invade the CNS during early development; as reviewed in (Kettenmann et al. 2011). Activation of microglia can be induced by systemic inflammatory factors, local CNS injury or chronic neurodegeneration and is accompanied by a change in their morphological phenotype (Cunningham et al. 2005). After brain trauma, microglia rapidly proliferate and migrate towards the lesion site, thereby establishing a barrier between the healthy and the injured tissue and constituting a first line host defense to the injury (Davalos et al. 2005). At the same time they can phagocytose the debris of damaged tissue, produce cytokines, chemokines and complement factors, upregulate MHC-II molecules and overall influence the inflammatory milieu in an either detrimental or beneficial way (Loane & Byrnes 2010; Nielsen et al. 2009). Astrocytes are the most abundant cell type in the CNS. Their primary role is to sustain homeostasis in the CNS and support the survival of neurons rather than to mediate inflammatory reactions. In response to injury astrocytes become hypertrophic, proliferate and migrate towards the borders of the lesion forming a reactive glial scar that isolates the injured area (McGraw et al. 2001). This sealing probably is a defence mechanism in order to protect the surrounding tissue from the damaging consequences. However, astroglial scarring can impede axonal growth and regeneration and thereby negatively affect outcome (Floyd & Lyeth 2007; Ridet et al. 1997; Shechter et al. 2011). Also, it is now becoming increasingly evident that astrocytes, upon activation by inflammatory factors, serve several auxiliary immune functions such as the production of cytokines and chemokines (Brambilla et al. 2005; McKimmie & Graham 2010).

1.1.2 Cytokines and Chemokines

Cytokines are small signaling molecules with inter-cellular activity that act upon cells and change their phenotype and activation status. Chemokines are small proteins that are produced and secreted by inflammatory cells to attract and recruit leukocytes. Cytokines are traditionally grouped into pro-inflammatory, such as IL-1b and TNF, and anti-inflammatory, such as IL-10 and TGF- β . However, for several cytokines, both deleterious and beneficial effects after TBI have been demonstrated. The dual role of cytokines with conflicting results between studies led to the use of the term “double-edged sword” to describe the role of the immune system in TBI (Morganti-Kossmann et al. 2002). The role of cytokines and chemokines in TBI has been reviewed in (Helmy, A. et al. 2011) and (Ziebell & Morganti-Kossmann 2010).

1.1.3 Complement Activation

The complement system is a highly conserved molecular cascade that is important mainly in innate but also in adaptive immunity. It is synthesized by soluble molecules that are produced mainly in the liver and circulate in the blood. These molecules are widely involved in the chemotaxis of inflammatory cells and in direct tissue/cell damage. Activation of the complement system is induced by distinct trigger mechanisms that are used to distinguish the three different subpathways in the cascade: the classical pathway, the alternative pathway and the mannose-binding lectin pathway. All three pathways subsequently lead to the final formation of the membrane attack complex (MAC); for review see (Gasque 2004; Ricklin et al. 2010). Complement factors do not cross the intact BBB under physiological conditions, but they can enter and exert their functions in the injured CNS. Several studies have also demonstrated that all types of brain cells can produce complement factors with their subsequent deleterious or beneficial involvement in the local inflammatory response; for reviews see (Brennan et al. 2012; Helmy, Adel et al. 2011; van Beek et al. 2003; Yanamadala & Friedlander 2010).

The production and involvement of complement mediators such as C3, C5a, CD55, CD59 and clusterin has been demonstrated in experimental TBI (Bellander et al. 1996; Leinhase et al. 2006b; Stahel et al. 2000). Furthermore, a number of studies have been performed utilizing genetically engineered animals or pharmacological intervention to elucidate the role of complement factors in TBI. Thus, following brain injury C3 and C5 null mice displayed an attenuation of the inflammatory response, and C3 and C4 null mice exhibited a better motor outcome, while absence of the complement inhibitor CD59 exacerbated neuropathological outcome (Sewell et al. 2004; Yang et al. 2006; You et al. 2007). Factor B null mice, lacking a functional alternative pathway, showed a significant reduction of neuronal cell death and administration of anti-factor B antibody attenuated inflammation and apoptosis (Leinhase et al. 2006a; Leinhase et

al. 2007). Administration of C1-inhibitor improved the motor performance and reduced the contusion volume of brain injured mice (Longhi et al. 2009).

In human TBI, Bellander et al found increased immunoreactivity against C1q, C3, C4, C3b, C3d and C5b-9 on neurons in the penumbra region of the contused brain tissue 2-82 hours after injury (Bellander et al. 2001). Furthermore, increased levels of C3, factor B and MAC have been demonstrated in the cerebrospinal fluid (CSF) of TBI patients (Bellander et al. 2011; Kossmann et al. 1997; Stahel et al. 1998; Stahel et al. 2001). Taken together, these human studies combined with the experimental data clearly point towards detrimental effects of a dysregulated complement system after TBI.

1.1.4 Major Histocompatibility Complex (MHC)

The main functional role of MHC class I and class II molecules is to present peptides on the cell surface to CD8+ and CD4+ T cells, respectively. It is generally stated that peptides originating from intracellular sources are presented by MHC class I molecules in most cells of the body, while those originating from extracellular sources are presented by MHC class II molecules in specialized antigen presenting cells (APCs). However, some mechanisms, such as cross-presentation and autophagic degradation of peptides, have recently been described so that class I molecules present exogenous peptides and class II molecules present endogenous peptides, respectively; reviewed in (Crotzer & Blum 2010; Kurts et al. 2010; Neefjes et al. 2011; Vyas et al. 2008). Human MHC molecules are called human leukocyte antigens (HLA) and are equivalent to the H-2 molecules in mice and RT1 molecules in rat. The rat class II molecules RT1B and RT1D are equivalent to human HLA-DQ and HLA-DR, respectively. MHC genes are the most polymorphic genes present in the genome of every species (Ting & Trowsdale 2002). Class I molecules are expressed on all nucleated cells, while class II are expressed on professional APCs such as dendritic cells, macrophages and B lymphocytes. Each class II molecule consists of two integral membrane proteins named α and β chains. The α and β chains of all classical class II molecules have the same overall conformation consisting of 2 extracellular domains: α_1 and α_2 , and β_1 and β_2 , respectively. The membrane-distal domains combine to form a single peptide binding domain that displays a high level of polymorphism and can bind multiple peptides with high affinity and low specificity (Fig.2).

The *RT1* complex is encoded on rat chromosome 20 and contains the classical *class I* region (*RT1-A*), the *class II* region (*RT1-B/D*), the *class III* region, which harbors genes that are implicated in innate immunity (e.g. *Tnf*, *C2*, *C4*, *Lta*, *Aif1*, *Ager*) and the nonclassical (*RT1-C/E/M*) *class I* region, which encodes numerous molecules that are not fully characterized (*xMHC*) (Hurt et al. 2004; Tuncel et al. 2012). It should also be

noted that the rat RT1^c haplotype expresses two *RT1A* (*MHC-I*) regions, while the RT1^{Av1} only one (Joly et al. 1996).

MHC class I and II antigen presentation is increased in the contused and pericontusional tissue following both experimental and human TBI (Engel et al. 2000; Holmin et al. 1995; Holmin et al. 1998; Oehmichen et al. 2009; Schwab et al. 2001). The presence of MHC-II positive microglia has been also demonstrated along the corticospinal axis also at a distant site from the initial injury (Nagamoto-Combs et al. 2010). Zhang et al has shown that the up regulation of MHC class II molecules can be attenuated by administration of the anti-inflammatory drug FTY720 after brain contusion (Zhang et al. 2007).

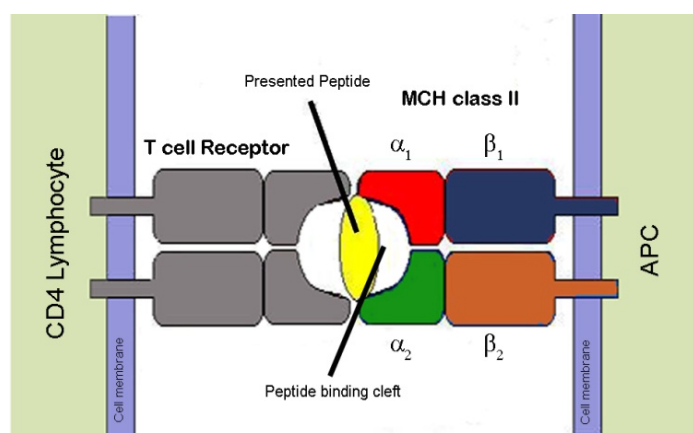


Figure 2. Schematic illustration of the MHC class II molecule architecture and peptide presentation to CD4 lymphocytes.

Apart from the above mentioned studies, no data up to date exist with regards to the regulation or effects of increased MHC-II presentation in head injury. One reason for this could be that the inflammatory reaction following TBI is considered to involve mostly the innate and less so the adaptive arm of the immune system.

1.2 OXIDATIVE STRESS

1.2.1 Oxidants

1.2.1.1 Free Radicals

Oxidative stress occurs when oxygen free radicals are generated in excess through the reduction of oxygen. A free radical is defined as any molecular species capable of independent existence that contains a number of unpaired electrons. Free radicals are shown to be involved in the pathophysiological cascades of many CNS diseases,

including traumatic brain and spinal cord injury, stroke and chronic neurodegenerative diseases. Increased production of free radicals in CNS diseases is aided by the fact that the brain consumes about twenty percent of the total amount of oxygen in the body and also displays the highest oxygen metabolic rate of any organ in the body (Zauner et al. 2002).

Two major categories of free radical species are the reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS include the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^{\cdot}), while RNS include nitric oxide (NO) and peroxynitrite ($ONOO^{\cdot}$). Both the superoxide and the hydrogen peroxide radical are poorly oxidizing species, but they can generate the highly reactive hydroxyl radical through the Fenton and Haber-Weiss reactions. These reactions are catalyzed by free iron Fe (III) that is produced in the brain by the breakdown of hemoglobin or its release from intracellular ferritin. The hydroxyl radical can also be generated by the reaction of superoxide with nitric oxide and in this reaction peroxynitrite is also formed.

The generation and role of free radicals have been demonstrated in numerous studies after TBI. Formation of superoxide and hydroxyl radicals in animal models of TBI induces various chemical and pathophysiological processes that lead to BBB dysfunction and mitochondria damage, as well as oxidative injury of DNA, proteins and lipids (Clark et al. 2001; Hall et al. 1993; Hall et al. 1994; Kontos & Povlishock 1986; Kontos & Wei 1986; Smith et al. 1994; Zhang et al. 2012). Furthermore, up regulation of all three nitric oxide synthases (NOS: endothelial, neuronal and inducible) occurs after TBI as well as the formation of peroxynitrite (Cobbs et al. 1997; Gahm et al. 2000; Gahm et al. 2002; Hall et al. 2004). Substantial information about the role of free radicals in TBI has been also gained from experimental and clinical studies with antioxidant pharmacological agents; reviewed in (Bains & Hall 2012).

1.2.1.2 Lipid Peroxidation

Lipid peroxidation occurs when ROS target lipids to form lipid radicals (L^{\cdot}). Once lipid radicals are produced, they react with O_2 to form peroxy radicals (LOO^{\cdot}). Peroxy radicals attack fatty acids (LH) to produce lipid hydroperoxides (LOOH) and new lipid radicals (L^{\cdot}), which in turn generate new LOO^{\cdot} ; in this way the reactions propagate for a number of cycles to produce high numbers of LOOH (Fig. 3). Lipid peroxidation results also in generation of highly reactive aldehydes such as malondialdehyde (MDA), acrolein, oxidized phosphatidylcholine, 4-oxo-2-nonenal and 4-hydroxynonenal (4-HNE, Fig. 3) (Adibhatla & Hatcher 2010).

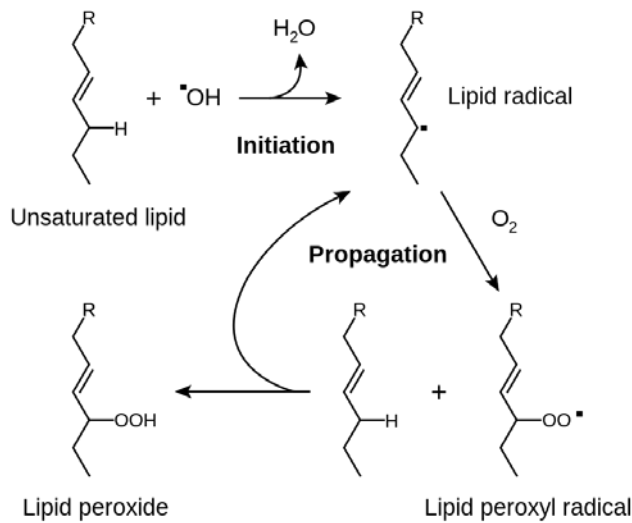


Figure 3. Schematic illustration of the lipid peroxidation cycle initiation and propagation.

Polyunsaturated fatty acids (PUFAs) are very susceptible to free radical attack, and since the CNS contains high levels of PUFAs, it is highly probable that lipid peroxidation occurs in the brain under oxidative conditions. Indeed, lipid peroxidation has been demonstrated in TBI by the thiobarbituric acid reactive substances (TBARS) assay or specific measurements of 8-epi prostaglandin F_{2a} (8-epi PGF_{2a}) or F_2 -isoprostane. The TBARS assay measures TBA-reactive substances such as MDA and has been widely used in experimental TBI research (Santos et al. 2005; Saraiva et al. 2012; Toklu et al. 2009). However, the TBARS test is not specific and direct assays with HPLC are recommended to define the exact levels of MDA. TBARS concentrations and MDA levels are increased in the CSF during the first days of TBI and have been found to correlate to the severity of the injury (Bochicchio et al. 1990; Kasprzak et al. 2001). F_2 -isoprostanes, one group of lipid peroxidation products derived from arachidonic acid, are initially formed *in situ* on membrane lipids and released by phospholipases in biological fluids (Morrow et al. 1990). F_2 -isoprostane has been detected in the CSF of brain injured patients where it displays an early peak at 24 hours, is higher in males compared to females, and is reduced by hypothermia (Bayir et al. 2002; Bayir et al. 2004; Bayir et al. 2009; Clausen et al. 2012; Varma et al. 2003). Increased levels of 8-epi PGF_{2a} have also been found at 6 and 24 hours after TBI in rats (Tyurin et al. 2000). Although there are no reports about the presence of 4-HNE in human TBI, in the experimental setting it has been shown to be increased early after TBI (Ansari et al. 2008; Gilmer et al. 2010; Readnower et al. 2010; Shao et al. 2006). Furthermore, antioxidant therapies against toxic pathways involving 4-HNE display a neuroprotective effect (Itoh et al. 2010; Sharma et al. 2010).

1.2.2 Antioxidants

The endogenous antioxidant defense system of the CNS is constituted by low molecular weight molecules such as glutathione, ascorbate and α -tocopherol and antioxidant enzymes such as super oxide dismutase (SOD), catalase, glutathione peroxidases (GPXs) and glutathione S transferases (GSTs).

1.2.2.1 Glutathione

Glutathione consists of a tripeptide of glutamate, cysteine and glycine and is characterized by a reactive thiol group and a γ -glutamyl bond. It is the most abundant endogenous non-protein thiol antioxidant present in all the cells. Under physiological conditions glutathione exists mainly in its reduced form (GSH) and only in a very small amount in the oxidized state (GSSG). An increased GSSG/GSH ratio can be used as a sign of oxidative stress. GSH can directly scavenge free radicals or act as a co-substrate in the enzymatic reduction of H_2O_2 and products of lipid peroxidation such as lipid hydroperoxides and 4-HNE. GSH can then be regenerated from GSSG by the reaction catalyzed by glutathione reductase (GR), which transfers electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to GSSG (Fig. 4A); for a review see (Dringen 2000).

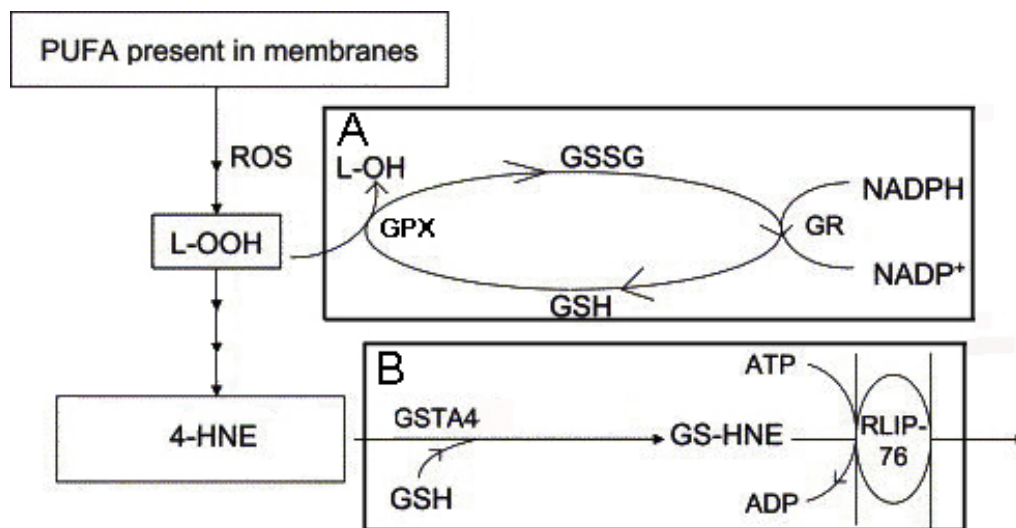


Figure 4. Glutathione metabolism pathways: Lipid peroxides (L-OOH) and 4-HNE are produced during peroxidation of polyunsaturated fatty acids (PUFA) by reactive oxygen species (ROS). A) Reduced glutathione scavenges lipid peroxides to produce oxidized glutathione (GSSG). This reaction is catalyzed by glutathione peroxidase (GPX). GSH is then regenerated from GSSG with the use of NADPH. B) Reduced glutathione (GSH) scavenges 4-HNE to produce GS-HNE, a reaction catalyzed by GSTA4. GS-HNE is then exported out of the cells by the Ral-interacting protein-76 (RLIP-76); modified from (Awasthi et al. 2004).

After experimental TBI, GSH levels in brain tissue decreased in the first days with lowest levels at 24 hours and with a concomitant increase in the glutathione oxidation ratio (GSSH/GSH) of 400% (Ansari et al. 2008; Chen et al. 2007; Tyurin et al. 2000). In infants and children with TBI, GSH levels in the CSF increased initially at day 1 and then decreased at days 5–7 as compared to controls. This was suggested to reflect an initial release of GSH into CSF from injured cells followed by a progressive decrease in cell antioxidant capacity (Bayir et al. 2002).

1.2.2.2 Antioxidant Enzymes

The antioxidant defense enzymes can be categorized in two groups based on the substrate: 1) the “first-line defense” enzymes such as SOD, catalase and GPXs that directly scavenge free radicals and 2) the “second-line defense” enzymes such as GPXs and GSTs that scavenge highly toxic molecules produced by free radical attack on DNA, proteins or lipids. SOD catalyzes the conversion of O_2^- to H_2O_2 and catalase forms water and molecular oxygen from H_2O_2 . GPXs are selenium containing enzymes that can both catalyze the reduction of H_2O_2 and the reduction of organic hydroperoxides to water or corresponding alcohols. They can therefore be a part of both the “first” and “second-line” antioxidant defense system. GPXs utilize reduced glutathione (GSH) as an electron donor (Fig. 4A).

The activity of SOD was reduced early after experimental brain injury, suggesting a decrease in the tissue antioxidant capacity (Ansari et al. 2008). Also, overexpression of SOD-1 in transgenic mice resulted in both acute and long-term beneficial effects after brain trauma (Mikawa et al. 1996). Both increased and decreased GPX and catalase activity has been reported in experimental TBI (Ansari et al. 2008; Goss et al. 1997; Kerman et al. 2012).

The GSTs comprise a complex and widespread enzyme superfamily that catalyzes the conjugation of exogenous and endogenous electrophilic substrates to glutathione. The classification of GSTs is based on a variety of criteria based on their amino acid sequence, location in the cell and substrate specificity as well as their immunological, kinetic and structural properties. Mammalian GSTs are highly polymorphic and can be divided in 7 subclasses: Alpha, Mu, Pi, Sigma, Theta, Omega, and Zeta; reviewed in (Sheehan et al. 2001). They are involved in multiple pathways such as in the clearance of carcinogens and thereby cancer pathogenesis, in drug resistance mechanisms, and they also act as protectors against oxidative stress by detoxifying highly toxic products of lipid peroxidation such as 4-HNE; reviewed in (Balogh & Atkins 2011; Landi 2000; Townsend & Tew 2003). 4-HNE can spontaneously react with the highly abundant GSH molecules in a reaction termed Michael addition. However, the reaction, when catalyzed by GSTs, can be 600 times

faster than the spontaneous reaction. It is now established that, of all the GSTs, Gsta4 has by far the highest enzymatic capability of reducing 4-HNE (Fig. 3B) (Balogh & Atkins 2011; Engle et al. 2004; Siems & Grune 2003).

1.3 NEURONAL DEATH AND AXONAL INJURY

Neuronal death in TBI starts immediately during the injury with the mechanical disruption of the brain tissue. However, an important feature of TBI is that a high degree of the neuronal damage occurs in a secondary phase which starts directly after the initial injury and continues for up to a year or even longer (Williams et al. 2001). Criteria such as cell morphological changes, type of death inducers, specific molecular mechanisms and enzymatic-dependence have all been used to classify neuronal cell death after TBI; as reviewed in (Stoica & Faden 2010). Based on morphological criteria, two predominant forms of cell death have been described in head trauma: necrosis and apoptosis. However, autophagic death has also been described and there is also evidence that necroptosis might also be a form of neuronal cell death after TBI (Luo et al. 2011; Wang et al. 2012). Necrosis, originally believed to be the only form of cell death in TBI, is not energy dependent, occurs when the cell is severely damaged and is characterized by cell and organelle swelling, disruption of membranes and chromatin digestion. Apoptosis, originally described in 1972 by Kerr et al, is a term used to morphologically describe a programmed cell death mechanism that induces cell shrinkage, nuclear condensation, membrane blebbing and the formation of membrane vesicles that enclose cellular components (Kerr et al. 1972). During the last two decades, programmed cell death has been extensively described after TBI and several aspects of the molecules that induce or propagate apoptotic cell death have been characterized; among many studies (Keane et al. 2001; Newcomb et al. 1999; Ng et al. 2000; Rink et al. 1995; Wennersten et al. 2003) and as reviewed in (Raghupathi 2004; Wong et al. 2005). It has to be emphasized that both inflammation and oxidative stress are key factors in neuronal cell death (Fatokun et al. 2008; Griffiths et al. 2009).

Axonal injury in TBI is the predominant feature of a separate type of brain trauma: diffuse axonal injury (DAI). The term DAI is used to describe the histological features of axonal injuries that are mainly caused by head trauma where rapid acceleration and deceleration of the brain occurs. However, axonal injury can also be observed in focal injuries as a delayed axonopathy and/ or axotomy that results from structural changes in the cytoskeleton of the axon, at the vicinity of the injured site, but also in areas remote from the initial impact. Neurofilaments comprise a group of neuron-specific intermediate structural proteins that are the predominant component of the axonal cytoskeleton and are therefore important in axonal injury studies. Neurofilaments (NFs) are composed of three subunits based on their molecular weight: a) light (NFL),

b) medium (NFM) and heavy (NFH). Changes in the amount and alterations in the normal structure of NFs have been described after TBI. Studies with electron microscopy have shown that the predominant histological features of axonal injury, axonal bulbs and swellings, contain accumulated and misaligned NFs (Christman et al. 1994; Posmantur et al. 1994; Posmantur et al. 2000). Also, NFH is detected in the CSF of patients with TBI and in some studies correlate to the severity of DAI and outcome (Siman et al. 2009; Zurek et al. 2011; Zurek & Fedora 2012).

1.4 TREATMENT

A significant part of neuronal death in TBI occurs in the secondary phase and this provides a therapeutic time window for neuroprotective treatment. At present, treatment of TBI consists of methods aimed to decrease raises in intracranial pressure and optimize cerebral perfusion to improve the oxygenation of the brain. As of today no pharmacological treatment with proven effect exists. This is in spite of great efforts directed at finding effective pharmacological agents. Many drugs have shown promising results in experimental TBI, but translation to humans has failed. Two widely acknowledged reasons for this are the faulty design of clinical trials and the fact that the complexity of human brain trauma cannot be mimicked by one single experimental TBI model. Other factors such as genetic predisposition and neurorehabilitation might also play a role; reviewed in (Maas et al. 2010a; Marklund & Hillered 2011). This has led to new recommendations as to the design and methodologies that should be used in future TBI clinical trials (Maas et al. 2010b). On the other hand, even if treatment has failed in the clinical context, the use of chemical compounds and pharmacological agents in experimental TBI has provided valuable information about the secondary injury pathways and their role regarding the outcome. It has to be emphasized that many of the drugs used in these studies target inflammatory and oxidative pathways; reviewed in (Marklund et al. 2006).

1.5 INFLUENCE OF GENETIC BACKGROUND

Recovery after TBI is variable even for patients with similar clinical characteristics and injury severity. Prediction of outcome is of major importance when taking decisions about the treatment of patients with TBI, particularly those with moderate to severe injury that are treated in intensive care units. The prognostic value of factors such as age, clinical severity and imaging characteristics of the injury are well studied and are thus used in predictive models. However, the relation of other factors to TBI outcome is less well identified (Lingsma et al. 2010). It is now becoming increasingly realized that the individual's genetic predisposition also contribute to the recovery and

outcome after TBI. This has recently been highlighted in several review articles (Dardiotis et al. 2010; Dardiotis et al. 2012; Diaz-Arrastia & Baxter 2006; Jordan 2007; Kurowski et al. 2012; Weaver et al. 2012; Wilson & Montgomery 2007).

Detailed data about the effect of genetic background on secondary injury cascades and outcome in experimental TBI is increasing in the last years, but despite this it is still scarce compared to other fields in neuroscience. Fox et al has demonstrated differences in behavioural outcome in three mouse strains while Witgen et al found no differences in hippocampal neuronal survival between two mouse strains differing at multiple loci in chromosome 4 (Fox et al. 1999; Witgen et al. 2006). Strain differences in the response to TBI have also recently been reported between Sprague–Dawley and Fisher rats, with the latter displaying higher intracranial pressure, more seizure activity and worse motor deficits, but a better cognitive performance (Reid et al. 2010). In another study, Sprague–Dawley rats had a speedier behavioural recovery compared to Long–Evans rats (Tan et al. 2009). Finally, differences in immune cell infiltration and neurodegeneration have been demonstrated between the DA and PVG inbred rat strains (Bellander et al. 2010).

In human TBI, the most extensively studied polymorphism is in the apolipoprotein E (APOE) gene. Apolipoprotein E is a 34 kilodalton protein that transports lipids between tissues and cells but also has been implicated in many other biological pathways (Kim et al. 2009; Mahley & Rall 2000). In humans APOE is encoded by a single gene on chromosome 19 and three common allelic variants ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) which yield three distinct isoforms (APOE2, APOE3, and APOE4, respectively). The APO $\epsilon 3$ allelic variant is the most frequent in caucasian populations (Corbo & Scacchi 1999), while the APO $\epsilon 4$ allelic variant is associated with Alzheimer's disease (Naj et al. 2011). The fact that TBI leads to amyloid deposition in the brain and increases the risk for developing Alzheimer's disease led to further studies of the role of APOE in TBI; reviewed in (Sivanandam & Thakur 2012). Several studies using human APOE transgenic or knockout mice in models of TBI have shown an effect of the $\epsilon 4$ allelic variant in the inflammatory response, oxidative stress, oedema formation, amyloid deposition and outcome (Chen et al. 1997; Crawford et al. 2009; Hartman et al. 2002; Lomnitski et al. 1997; Lynch et al. 2002). In parallel, association studies in human TBI patients have shown *APOE4* to be connected to various injury parameters and a poor outcome after TBI. However, other studies have failed to replicate these findings. A meta- analysis of all these studies indicated that the *APOE4* variant does not influence the initial injury parameters but is associated with a poorer outcome at 6 months after the trauma (Zhou et al. 2008).

A smaller number of association studies have suggested an association of TBI outcome to polymorphisms in genes shown in table 1. Other studies show negative results for polymorphisms in *interleukin-1a*, *interleukin-1b*, *interleukin-6* and *NEFH*

genes (Johnson et al. 2006; Tanriverdi et al. 2006). These studies point to specific genes influencing the pathology and/or outcome after TBI. However, several concerns exist as to the methodology and design used in such investigations since they allow a high risk for false positive or negative results (Diaz-Arrastia & Baxter 2006; Hardy 2002; Sawcer 2010). This stems from the fact that the low number of patients included in association studies do not reach the necessary statistical power needed to confidently describe genetic influences. Furthermore, variation in patient selection, different ethnicities in the studied population, failure to control for confounders (for example age, injury severity, sex and treatment approaches) and variability in outcome parameters limit the ability to replicate these studies (Dardiotis et al. 2012). A limitation of these studies also emerges from the fact that only one gene polymorphism “of interest” is examined and this method cannot unravel additive effects or other gene polymorphisms of possibly stronger effect (Comings 2003).

<i>Gene</i>	<i>Number of patients</i>	<i>Outcome Parameter</i>	<i>Study</i>
<i>ACE</i>	73	Cognition	(Ariza et al. 2006)
<i>BDNF</i>	109	Cognition	(Rostami et al. 2011)
<i>BDNF</i>	168	Executive function	(Krueger et al. 2011)
<i>BDNF</i>	75	Cognition	(McAllister et al. 2012)
<i>CACNA1A mut</i>	2 families	Cerebral oedema	(Kors et al. 2001)
<i>COMT</i>	113	Executive function	(Lipsky et al. 2005)
<i>DRD2</i>	39	Cognition	(McAllister et al. 2005)
<i>Interleukin-1b</i>	151	Brain hemorrhage	(Hadjigeorgiou et al. 2005)
<i>Interleukin-1b</i>	69	GOS	(Uzan et al. 2005)
<i>Interleukin-6</i>	77	Survival	(Dalla Libera et al. 2011)
<i>Neprilysin</i>	81	Amyloid-beta plaques	(Johnson et al. 2009)
<i>NOS3</i>	51	Brain perfusion	(Robertson et al. 2011)
<i>PARP-1</i>	191	GOS	(Sarnaik et al. 2010)
<i>P53</i>	90	GOS	(Martinez-Lucas et al. 2005)

Table 1. Studies up to date suggesting an association of gene allelic variations with various outcome parameters after traumatic brain injury. (mut = mutation, GOS = Glasgow Outcome Scale)

The best way to tackle this problem is by designing genome-wide association studies in well powered clinical materials, an approach that has revolutionized the field of genetics in other diseases. Identification of low risk loci that influence susceptibility to disease have thus provided important information in neurological diseases such as multiple sclerosis (MS), Parkinson’s and Alzheimer’s disease (Bras et al. 2012; Gandhi & Wood 2010; Sawcer et al. 2011). Although, a similar approach in TBI is feasible in

principle, the heterogeneity of the disease, including multiple and insufficient outcome measures, and lack of large size material collection make this difficult to achieve in a short term perspective. An alternative approach is to use animal disease models combined with forward genetics in order to identify genetic factors and address some of the above mentioned problems. The results of animal studies can reveal new candidate genes and point towards certain pathways that in turn can be tested for a possible relevance in human TBI.

1.6 VENTRAL ROOT AVULSION AS A TOOL IN GENETIC STUDIES OF NEUROINFLAMMATION AND NEURODEGENERATION

Root avulsion in the rat mimics a specific type of plexus injury in humans and has thus been used to study surgical regenerative approaches in this disease (Carlstedt 2008). Avulsion of ventral roots transects motor axons at the very exit from the cord, thus at the interface of the peripheral and central nervous systems. The induced trauma results in a retrograde response in the spinal cord which is characterized by local activation of inflammatory cells and molecules, up or down regulation of neurotrophic factors, gliosis and motorneuron degeneration (Hammarberg et al. 1998; Koliatsos et al. 1994; Lundberg et al. 2001). The VRA model is simple and highly reproducible with a low degree of inter-individual variation. For this reason, it has been used with success as a model in forward genetics in order to identify genetic loci that regulate neuroinflammatory and neurodegenerative pathways in the CNS.

Studies in inbred rat strains showed a sizeable degree of strain-dependent regulation of inflammation, gliosis and motorneuron degeneration after VRA (Lundberg et al. 2001). This regulation was mostly accounted by variability in *non-MHC* genes (Olsson et al. 2000). Following from this, an F2 cross between the strain with highest and lowest motorneuron degeneration (DA and PVG, respectively) but also with differences in the local inflammatory response such as MHC class II presentation, recruitment of T cells and glial activation, could disclose genetic loci that regulated these phenotypes. Two gene regions affecting neurodegeneration were detected: *Vra1* on chromosome 8 with a significant linkage peak and *Vra2* with a suggestive linkage and weaker effect on chromosome 5. Furthermore, MHC-II presentation was regulated by *Vra4* with a very strong linkage peak on chromosome 10 and a significantly weaker effect of the MHC gene complex itself (Lidman et al. 2003). Subsequent studies showed that the gene that accounted for the effect of *Vra4* on MHC-II presentation was the class II transactivator (*Ciita* or *Mhc2ta*) (Swanberg et al. 2005). In addition, the effect of *Ciita* could also be replicated in the model of experimental autoimmune encephalitis (EAE) (Harnesk et al. 2008). Also, polymorphism in the type III promoter of the *Ciita* gene was associated with increased susceptibility to MS, providing evidence for a translational potential of the VRA-

genetic approach in neuroinflammatory and neurodegenerative CNS diseases
(Swanberg et al. 2005).

2 AIMS OF THIS THESIS

The purpose of this thesis was to study the impact of inherent genetic predisposition and further characterize gene regions that regulate inflammation and neurodegeneration in TBI as studied in the rat. The specific aims of this thesis are categorized as follows:

- 1) Study the impact of genetic variability occurring among inbred rat strains on the biological response after TBI in the rat.
- 2) Study the relative contribution of MHC and non-MHC mediated genetic effects on the biological response to TBI, with special emphasis on MHC-II presentation.
- 3) Characterize and identify candidates in the *Vra1* locus in a model of neurodegeneration.
- 4) Characterize the effect of the *Vra1* locus in experimental TBI.

3 METHODOLOGICAL CONSIDERATIONS

3.1 CHOICE OF EXPERIMENTAL MODELS

3.1.1 Experimental Traumatic Brain Contusion (I, II and IV)

In the present thesis we used the open skull weight drop injury (WDI) model, originally described by Feeney et al (Feeney et al. 1981), and we modified it as to produce a contusion in the rat parietal cortex above the hippocampus. The contusion device is composed of a stainless steel tube, a free falling weight and a piston attached to a stereotactic device. The weight moves freely in the tube that has air holes on the sides in order to avoid air compression. The anesthetized rat is placed on a stereotactic frame and a craniotomy is performed, 3 mm behind and 2.3 mm lateral to the bregma, with great care not to damage the dura. The footplate of the piston is positioned vertically on the exposed dura and the device is then lowered. The piston can freely be pushed back in to the tube by the exposed cortex/dura. The weight is then freely dropped from 7 cm and hits the piston that compresses the tissue at a maximum depth of 3 mm (Fig. 5). In sham operated control animals, surgery is finished after the craniotomy.

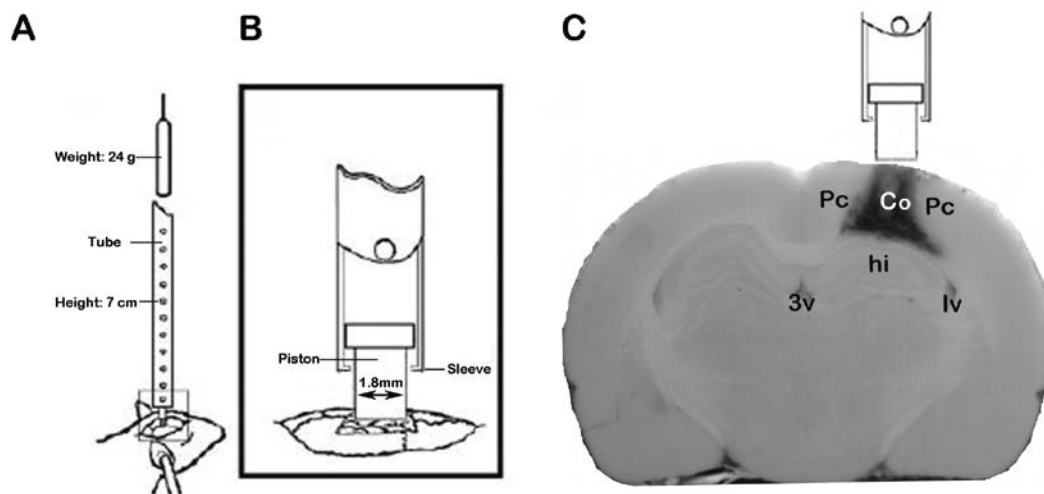


Figure 5. Schematic illustration of the contusion device and the produced traumatic injury: A) the weight-drop device composed of the weight (24 g), the tube and the piston and positioned above the exposed dura; B) a magnified picture of the marked frame in A showing the cylindrical piston, with a diameter of 1.8 mm, moving freely in the tube and being able to compress the exposed brain tissue; C) the lesion produced by the weight-drop device with the contused cortex (Co), the pericontusional tissue (Pc), the underlying hippocampus (hi) and the third (3v) and lateral ventricle (Iv).

Primary injury in human TBI is usually caused by two major mechanical phenomena: 1) a direct mechanical force that causes skull distortion and an underlying brain contusion or/and a contusion contralateral to the impact (contrecoup), 2) an indirect mechanical force that accelerates the brain tissue with relation to the skull or other surrounding structures and causes tissue distortion and tearing (O'Connor et al. 2011). These mechanical phenomena produce a wide range of brain tissue and vascular injuries, which explains the finding that several types of injury usually coexist in human TBI, including contusions, subdural and epidural hematomas, axonal injury and subarachnoidal hemorrhage. Over the last decades a wide range of animal models of TBI have been produced. These models can be classified: 1) according to the characteristics of the mechanical force to static, dynamic/direct and dynamic/indirect (Cernak 2005) or 2) according to the characteristics of the lesion to focal, mixed, diffuse and complex models (Marklund & Hillered 2011). An example of a static model is a cranial nerve crush injury (Kiernan 1985) and of a dynamic/indirect model is a blast injury (Risling et al. 2011) while the most commonly used dynamic/direct models are shown in table 1.

	<i>Dynamic/Direct</i>	<i>Reference</i>
Focal	Controlled cortical impact (CCI)	(Dixon et al. 1991)
	Open skull weight drop injury (WDI)	(Feeney et al. 1981)
Mixed	Lateral fluid percussion injury (IFPI)	(McIntosh et al. 1989)
Diffuse	I/A “Marmarou” Model	(Marmarou et al. 1994)
	Central fluid percussion (cFPI)	(Dixon et al. 1987; McIntosh et al. 1987)
Complex	Combination of above models with hypoxia and/or hypotension	(Ishige et al. 1987)

Table 2. Examples of the most commonly used dynamic/direct experimental TBI models in the rat. The models are classified according to the characteristics of the lesion they produce. References for the first description of the model in the rat are given (for a review see Marklund and Hillered, 2011).

All of the above models replicate one or several aspects of human TBI but no single model can reproduce the heterogeneity of clinical TBI alone. However, by providing reproducibility and control of injury parameters, access to the injured tissue, large sample sizes and possibility to modify the genotype, they have altogether been essential for understanding the pathophysiologic mechanisms that follow human brain trauma and also for testing various treatment approaches. The WDI model is a focal dynamic/direct experimental model that produces a highly reproducible lesion resembling a human contusion (Holmin et al. 1995; Holmin et al. 1998). Some concerns about WDI have been raised as to the 1) craniotomy performed prior to the

trauma, which does not resemble the clinical setting of closed trauma (Flierl et al. 2009), 2) variation of the impact velocity and 3) the possibility of rebound trauma (O'Connor et al. 2011), the latter two as opposed to the similar CCI model. In this thesis a low height of 7 cm was used that minimizes both the variation of impact velocity and the possibility of a rebound effect. In addition, the WDI model is fast and pathophysiological changes are seen and can be studied in the contused cortex, the pericontusional cortex and the ipsilateral hippocampus and thalamus and at a lower degree in the contralateral hemisphere (Clausen & Hillered 2005; Holmin et al. 1995; Lewen et al. 1996).

3.1.2 Ventral Root Avulsion (III)

In the VRA model used in this thesis, the L3-L5 ventral roots have been torn away from the spinal cord resulting in a lesion at the border between the central and peripheral nervous system (PNS) (Fig. 6).

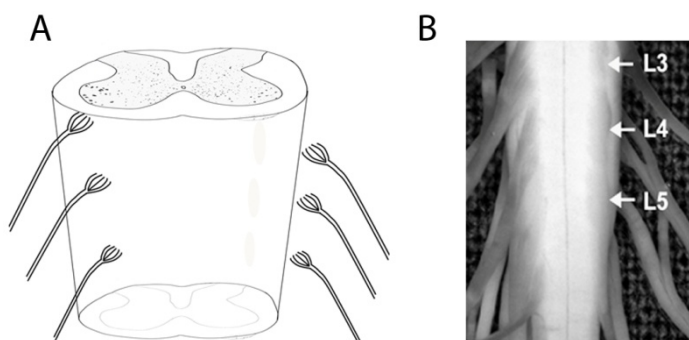


Figure 6. Schematic illustration in (A) and live image in (B) of the ventral root avulsion model where the L3-L5 ventral roots are pulled from the spinal cord (B adopted from (Lidman et al. 2003)).

The detachment of ventral roots initiates local pathophysiological mechanisms in the ventral horn of the spinal cord that subsequently lead to motorneuron degeneration. After VRA, the spinal cord was collected at different time points based on previous studies on the kinetics of glial reaction, inflammation and neurodegeneration (Piehl & Lidman 2001). Thus earlier time points (1-14 days) were used to assess pathophysiological pathways and a later time point (21 days) to assess the degree of neurodegeneration. Motorneuron loss was evaluated by counting the remaining motorneurons on the injured side of the spinal cord and comparing them to the number of neurons on the intact side. The motorneuron counting was initially performed on sections stained with cresyl violet. However, to avoid problems that arise from this type of profile counting, the counting methodology was later complemented with design-based stereology (see section 3.5.2).

The VRA model was chosen to complement the genetic studies in this thesis due to 1) high reproducibility with a low degree of inter-individual variation as compared to the

WDI, characteristics that are advantageous in animal genetic studies and 2) the long experience and extensive knowledge of this model in our laboratory.

3.2 HUMAN TRAUMATIC BRAIN INJURY BIOBANK (IV)

In paper IV, we used CSF from our human TBI biobank. In 2007, we started collecting blood for DNA analysis, serum and CSF from patients that were treated in the neurointensive care unit. Informed consent was obtained from relatives to the patient. Collection of clinical data was obtained from the patient's medical record. One hundred patients were included in the biobank until autumn 2010, when we started the analyses for 4-HNE in paper IV. By now, biological samples from approximately 170 patients are stored in the biobank. Unfortunately, we were not able to detect the targeted marker (4-HNE) in any of the CSF tested, but this biobank can now be used in subsequent analyses to translate results from this thesis in the human context.

3.3 METHODS FOR GENETIC DISSECTION

Studies on the contribution of genes and genetic loci on a disease phenotype can be performed by two approaches, forward or reverse genetics. The forward approach starts by identifying a disease phenotype, or quantitative trait, and thereafter tries to identify the specific genomic sequence(s), that account for the observed variation. The identified genomic region is termed a quantitative trait locus (QTL). In contrast, the reverse approach starts by identifying a specific gene variation/mutation and thereafter tries to find its contribution to a phenotype or biological mechanism. In the natural biological context, the former method is more likely to be successful to understand the genetic basis of common disease phenotypes. The main reason for this is that disease phenotypes are mostly affected by small/moderate contributions from several genes (i.e. in complex diseases) and single gene mutations are only in rare cases the cause of common disorders. In this thesis the forward approach was utilized since it is predicted and also supported from previous studies that quantitative TBI and VRA phenotypes are regulated by multiple genes with varying quantitative effects.

3.3.1 Inbred Rat Strains and Crosses (I-IV)

Genetic dissection of quantitative traits by forward genetics in animal models is performed by starting with two or more inbred rat strains that differ in a specific trait. Inbred rat strains are nearly identical and homozygotes at each locus in the genome.

Crossing of strains results in recombination and crossover between homologous chromosomes occurring during meiosis. Crossing of two inbred rat strains creates an F1 generation, where all individuals are identical heterozygotes at all loci. Further crossing of the F1 generation will result in an F2 intercross where all individuals are unique since recombination has occurred between the chromosomes (Figure 7). By determining the genetic composition and disease phenotypes in each of the F2 individuals, a statistical probability that a certain genetic region is linked to a variation in the phenotype can be established; in this way a QTL is identified.

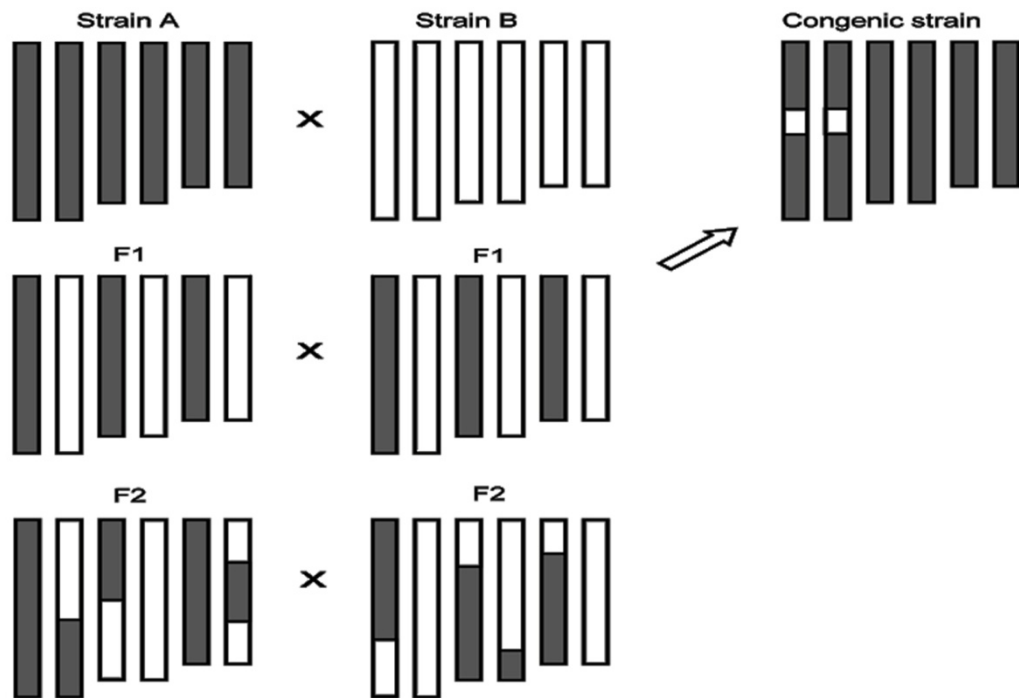


Figure 7. Schematic illustration of the forward genetics breeding strategy used in this thesis; as discussed in the text.

Subsequent and continuous intercrossing from the F2 generation will result in an advanced intercross line where more recombinations are accumulated and thereby smaller chromosome fragments can be identified as QTLs underlying the trait (Darvasi 1995).

In this thesis, we utilized two inbred rat strains, the Dark Agouti (DA) and Piebald Virol Glaxo (PVG), and crosses between them as shown in figure 7. These strains were chosen because of their differences in biological pathways and susceptibility to EAE and VRA. The DA strain is susceptible to EAE while the PVG strain is resistant and, similarly, the DA displays a stronger local inflammatory response and less motorneuron survival after VRA compared to the PVG.

3.3.2 eQTL Mapping (III)

Any trait can be mapped with linkage analysis and a particular approach that has emerged in recent years is the mapping of gene expression QTLs (eQTLs). The recent technological advancement enables analysis of the expression of several thousand mRNA transcripts using microarray chips, and these data can be analyzed with linkage analysis in a global gene expression profiling approach. Combination of forward genetics and microarray data and analysis with eQTL mapping can give information about the regulation of multiple transcript levels by multiple genetic locations. In such an analysis, when an eQTL encloses the physical location of the gene for that transcript, it is likely that the genetic variation resides in or close to the gene itself (cis-QTL). On the other hand, if an eQTL does not map to the physical location of the gene for that transcript, it is likely that the genetic variation is caused by other genes (trans-QTL) (Drake et al. 2006). An example of a trans-QTL could be a genetic variation in a transcription factor which affects many different genes located elsewhere in the genome. In paper III, we performed VRA and genotyping in a F2 generation followed by affymetrix analysis in order to study the linkage of the transcript differences to specific loci and genes.

3.3.3 Congenic Rat Strains (II-IV)

In a congenic strain, a small fragment of the genome of inbred strain B is introgressed in the genome of inbred strain A. Thus, strain B contributes to the background genome with this fragment in the resulting congenic strain. In this way, phenotypic differences that are found between the congenic and background strain A can be attributed to the genetic effect of the specific fragment originating from strain B. Congenic strains are produced by continuous backcrossing of the F1 generation with the inbred strain that is aimed to become the background strain (Fig. 7). Congenic strain production can be done in two ways: 1) the traditional approach by selecting the individuals containing the desired fragment for further backcrossing and 2) by the “speed congenic” method where the whole genome is genotyped and individuals are selected both on the desired fragment but also based on the lowest amount of contaminating genome from strain A in each generation (Wakeland et al. 1997). The first method is slower and it takes at least 10 generations to produce a congenic strain while the “speed congenic” method, although faster, it requires more effort in genotyping.

In this thesis four congenic strains were used: 1) the PVG-RT1^{av1} or PVG^{av1} congenic which is an MHC congenic with PVG background but with the MHC region on chromosome 20 introgressed from the DA strain (hereafter PVGA), 2) the PVG^{av1}.DA-*Vra4* congenic on PVG background with the *Vra4* locus on chromosome 10, that includes the *Ciita* gene, from DA, 3) the DA.PVG^{av1}-*Vra4* congenic on DA background

with the *Vra4* locus from PVG and 4) the DA.PVG^{av1}-*Vra1*-R5 on DA background with the *Vra1* locus on chromosome 8 from PVG.

Congenic rat strains are very valuable in forward genetics, when trying to confirm and study the effect of a QTL that has previously been identified by other methods (i.e. linkage analysis) to affect a disease phenotype. However, a disadvantage of this method is that it is very time consuming and sometimes virtually impossible to produce a “one-gene” congenics in order to study the effect of a single gene variation. In this way, reverse genetics, with the production of transgenic animals can be utilized to complement the information derived by congenic strains and to provide formal proof of the effect of a specific gene. However, it should be noted that transgenic animals that are produced “artificially”, cannot be used alone in the context of complex diseases when investigating natural polymorphisms that drive a disease phenotype.

3.4 EVALUATION OF PATHOPHYSIOLOGICAL PATHWAYS

3.4.1 mRNA Identification and Quantification (I-IV)

The regulation of the different effector molecules in various pathophysiological pathways is widely studied by the quantification of their mRNA levels. The tissue piece that was taken for mRNA quantification was composed of the contused tissue, the pericontusional cortex, the hippocampus and part of the thalamus, since these regions

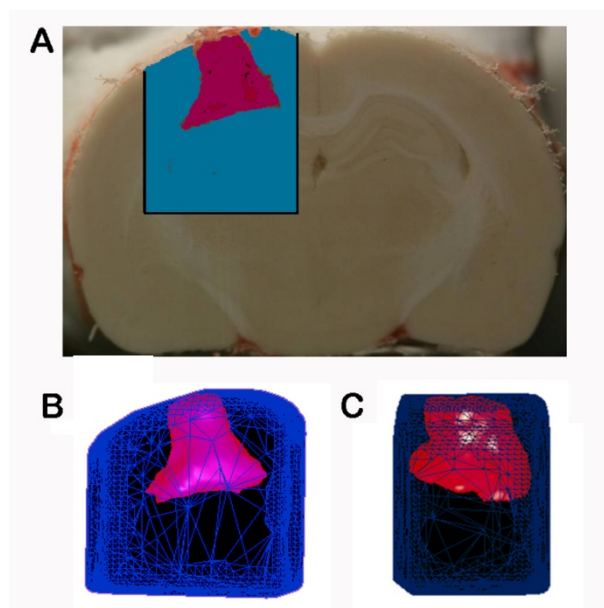


Figure 8. Schematic illustration of the tissue piece that was taken for mRNA quantification.

As seen in A it contains the contused tissue, the pericontusional cortex, the hippocampus and part of the thalamus. B and C show a 3D illustration in coronal and sagittal view respectively. The contused tissue is shown in red while the pericontusional tissue is shown in blue.

contain the strongest biological responses to injury in the WDI model (Fig. 8). We used two methods to identify and quantify mRNA levels: real-time quantitative polymerase chain reaction (qPCR) and Affymetrix arrays.

Real-time quantitative PCR is a simple, fast, accurate and reliable method for quantification of mRNA levels of specific molecules. However, a limitation of qPCR is that it does not provide information about the cellular localization of the molecules studied. After mRNA extraction from the tissue, RNA-dependent DNA polymerases called reverse transcriptase are used to create complementary DNA (cDNA). cDNA is then detected, amplified and quantified in “real-time” with the use of a fluorescent probe/primer (Heid et al. 1996). When studying expression of MHC-II and since the MHC region is highly polymorphic, appropriate care was taken to make sure that the primer did not display different affinities/avidities for the two *RT1B* haplotypes. Therefore, the *RT1Ba* primer was designed from a gene region that was identical between the two haplotypes (*c* and *av1*). A set of reference/housekeeping genes is used for normalization in order to compensate for differences in the amount of the starting tissue volume. After TBI, HPRT has been shown to be the most stable reference gene in the cerebral cortex while GAPDH is the most stable in the hippocampus (Cook et al. 2009). We have therefore used a combination of both HPRT and GAPDH as reference genes.

The development of chip based screening techniques during the last decade has enabled the study of the expression of all exons across the genome by using hybridization technology (Lockhart et al. 1996). We used Affymetrix Exon Arrays in our studies to obtain quantitative information about the whole transcriptome in the tissue, so called global gene expression profiling. The data were then uploaded in the Partek[®] software where they were normalized using Robust Multi-Array Analysis (RMA) to account for whole-chip expression levels (Irizarry et al. 2003). This allowed for further analysis in order to identify genes and pathways that were differentially regulated using Partek[®] and Ingenuity software[®].

3.4.2 Protein Identification and Quantification (I-IV)

Quantification of mRNA is not sufficient to provide information about disease related molecules as all mRNA is not transcribed into proteins and it is proteins that exert the real effect in biological responses. A combination of the qPCR and Affymetrix data with protein studies is therefore required. We have used four methods to identify and quantify proteins of interest: western blot and immunohistology, for tissue, as well as enzyme-linked immunosorbent assays (ELISAs) and Meso Scale Discovery (MSD[®]) Multi-Array, for CSF. All four methods utilize antibodies to identify and quantify the targeted proteins. Thus, it is of major importance to verify, as much as possible, the specificity of the antibodies used. Western blot is used for quantification of proteins

by size separation of the proteins and quantification of the band only of the right molecular weight; it can thereby discriminate between specific and unspecific signal. However, it does not provide any information about the tissue/cellular localization. This is done best with double/multiple-labeling immunofluorescence combined with analysis in a confocal microscope; for example with simultaneous staining of the protein in question together with a cellular marker protein. On the other hand, immunohistology is not considered a good method for quantification of proteins, in particular when profile counting and not stereological methodology is used. However, this problem can be overcome when large differences are expected/ seen in the tissues compared. Quantification should then be done by a blinded observer to avoid bias. ELISA is a frequently used, fast and reliable method for quantification of proteins particularly in body fluids (Engvall & Perlmann 1971). MSD[®] Multi-Arrays allows such a quantification to be performed on a smaller fluid volume and for multiple targets (Fu et al. 2010).

3.4.3 Quantification of Immune Cells (I-II)

In order to detect and quantify immune cells in the injured brain we used flow cytometry. This method has been widely used to quantify immune cells in the blood or dissociated spleen and only a few studies have attempted to quantify immune cells in the injured CNS by flow cytometry using fresh dissociated tissue. Flow cytometry is a quick and reliable method that has several advantages over traditional methods of assessing the cellular inflammatory response after CNS injury (Beck et al. 2010). Traditional analyses with immunohistology require preparation of tissue and manual counting of immune cells and offer semiquantitative data at best. Furthermore, the populations of blood-derived leukocytes can be rapidly quantified and distinguished from resident microglia. However, the use of flow cytometry to quantify immune cells in the CNS is complicated by lipid/myelin content and debris. These can interfere with antibody binding and decrease measurement sensitivity and accuracy. Several methods to overcome this problem have been described with the use of different gradient systems in order to get rid of the myelin (Stirling & Yong 2008; Tjoa et al. 2003). In this thesis, we used the Percoll gradient to separate cell populations from myelin/debris and the appropriate cell marker antibodies to distinguish and quantify the various cell populations.

3.5 EVALUATION OF INJURY OUTCOME AND NEURODEGENERATION

3.5.1 Contusion Volume (II)

The outcome of experimental brain contusion was assessed in paper II by quantification of the necrotic cavitation produced 18 days after the injury. Serial coronal sections were stained with hematoxylin-eosin and the area of the necrotic lesion and the ipsilateral to the injury hemisphere were quantified using an image analysis system. The percentage of lesion area for each section was calculated using the formula: (volume of necrotic lesion)/ (volume of ipsilateral hemisphere) x 100. The percentages of all areas were added in every animal to give an estimation of the lesion volume. Some concerns have been raised about this method because oedema was found in some studies to contribute to an inaccurate estimation of the lesion volume (Baskaya et al. 2000). However, the studies are contradicting and our measurements, done at 18 days after the injury, should not be influenced by oedema as it is considerably reduced at this time point (Elliott et al. 2008). Furthermore, the volume of necrotic cavitation has in several reports been found to correlate to behavioral deficits and this method is widely used to assess outcome in TBI (Clausen et al. 2005; Marklund et al. 2001).

3.5.2 Design-Based Stereology (III-IV)

In papers III and IV we used design-based stereology to estimate the degree of neuronal cell survival in VRA and TBI respectively. The term stereology was coined for the first time in 1961 at the “Foundation of International Society for Stereology” and is used to describe a set of methods that help us derive three-dimensional (3D) information from two-dimensional objects (2D), such as tissue sections. Stereology has been used since the ancient times in astronomy, for example in the heliocentric theory of Aristarchus, and has in the latest decades evolved to become the “state of the art” when counting structures such as cells in biological samples (Elias et al. 1971). It utilizes various sampling schemes, probes and mathematical quantification formulas to estimate the length, volume, surface or number of cells in a tissue sample. The term “design-based” means that the probes and sampling schemes are designed before the actual counting and this is done in such a way that no assumptions are made about the geometries of the cells being counted (Schmitz & Hof 2005; West 2001). Design based stereology has several very important advantages over profile tissue section counting. Although it is a time consuming procedure, it is the golden standard for making unbiased, accurate and precise estimations when evaluating cell structures from 2D tissue samples. Therefore, it enables the detection of small differences in the counted objects that are also statistically valid; for a review of the advantages of stereology see (Boyce et al. 2010) .

In this thesis, sections were cut perpendicular to the long axis of the tissue and they were sampled according to the principle of systematic random sampling (Fig. 9 A and B). The optical fractionator was used to count neurons with the use of the newCAST software (Visiopharm, Hoersholm, Denmark) (Fig. 4C). The subregion of the spinal cord or the hippocampus was delineated and the nucleoli of neurons were counted as they came into focus when the focus plane was moved downwards (Dorph-Petersen et al. 2001; West et al. 1991). Nucleoli were counted using the unbiased counting rules for the optical disector (Gundersen et al. 1988; West 1993). Neurons were identified by their heavily stained cytoplasm, large nucleus and single nucleolus. Motorneurons were identified by their large soma diameter, polygonal shape and prominent nucleus. The nucleolus was used as the counting unit since neurons have only one nucleolus (Bjugn & Gundersen 1993; Penas et al. 2009).

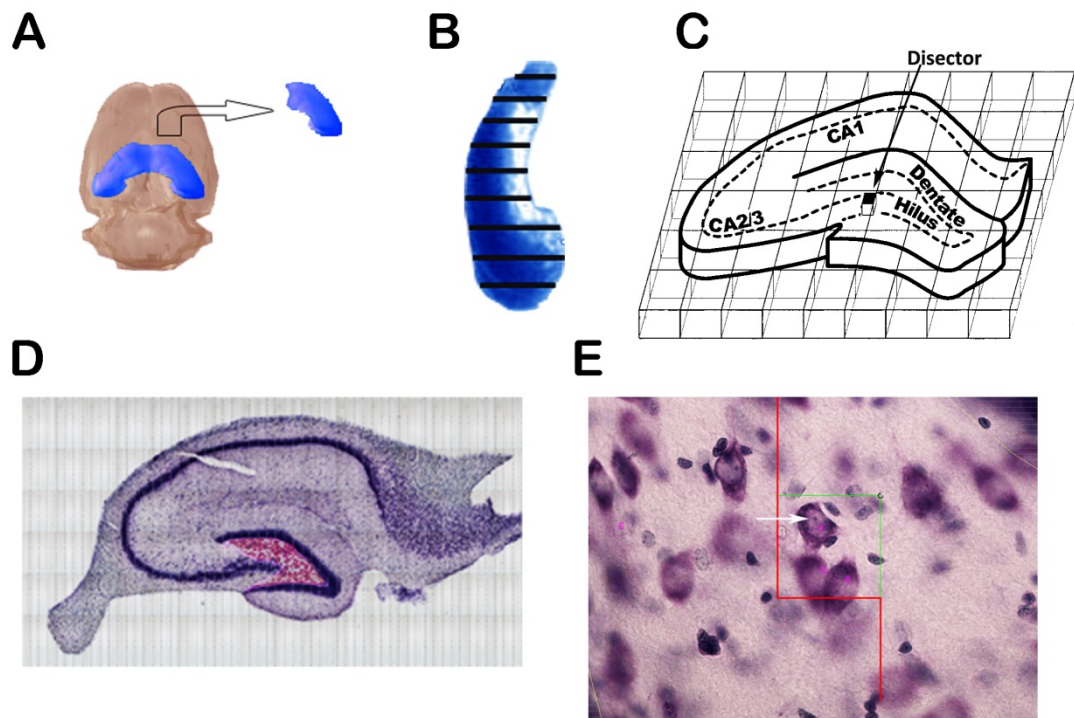


Figure 9. Schematic illustration of the design based stereology procedure used in paper IV to estimate the numbers of neurons in the hilus: A) The hippocampus ipsilateral to the injury was extracted from the brain B) The hippocampus was straightened and then sectioned on a vibratome in 65 µm thick sections (black lines). The first section was randomly chosen and every 15th section was kept for histological staining and counting of neurons in the hilus. C) An illustration of the optical disector principle, used to count cells in the thick hippocampus sections, is shown. Virtual counting boxes (disectors) will cover the hilus with a uniform distance between them. The edges of the disector are at a certain distance away from the surface to prevent bias due to surface tissue deformations. D) The area of interest where cell counting is performed is delineated (here the hilus shown in red). E) The virtual counting box (optical disector) is shown at a certain point on the z-axis. The nucleolus that comes in focus (white arrow) is counted. Nucleoli that would touch the green lines would be counted but not those that would touch the red lines.

4 RESULTS AND DISCUSSION

4.1 *NON-MHC* GENES REGULATE SECONDARY INJURY CASCADES AND OUTCOME

Strain dependent genetic differences have been recognized for many years to affect susceptibility to experimental autoimmune encephalomyelitis (EAE), the animal model of MS (Kornblum 1968). Susceptibility to EAE is hierarchically determined by genes in the *MHC-complex* itself but with additional influences also from *non-MHC* genes (Weissert et al. 1998). Studies in non-autoimmune CNS and PNS injury models identified both *MHC* and/or *non-MHC* genes to regulate the phenotypic outcome (Bellander et al. 2010; Birdsall Abrams et al. 2007; Dominguez et al. 2008). In paper I, II and IV we studied the impact of *non-MHC* genes on secondary injury cascades and outcome after TBI by performing WDI on the inbred rat strains DA and PVGA that share the same MHC complex (RT1^{av1}) (Fig. 10 A).

Using affymetrix we performed global expressional profiling of injured brains at 1 day post-injury (1 DPI). We found that variation in the expression of around one third of the more than 27000 examined transcripts could be accounted for by non-MHC genes, if differences between naïve animals were also included. Analyses of the microarray data with the Ingenuity software showed that inflammation was one of the top biological functions regulated by both strain and injury. Analyses were also performed to search for pathways differing between the two strains after TBI. This revealed glutathione metabolism to be the top regulated pathway, while the complement system was the top regulated inflammatory pathway. In parallel, our experiments in a model of nerve axotomy (VRA) suggested *Gsta4* to be a candidate gene regulating neurodegeneration between the two strains. Subsequent studies were thus focused on inflammatory (paper I and II) and glutathione (paper III and IV) related mechanisms suggested to be regulated by *non-MHC* genes.

Immune cell influx and activations was studied using flow cytometry. The DA rat displayed higher numbers of infiltrating granulocytes and NK cells (in particular CD161 bright) in the brain at 1 DPI. Although the mean numbers of monocytes/macrophages were also higher in the DA strain, it did not reach statistical significance, while the number of T lymphocytes did not differ. At the same time-point a stronger host tissue response, as measured by numbers of activated microglia, was also seen in the DA strain. At a later time-point (6 DPI) the numbers of blood derived leukocytes did not differ between the strains except for macrophages that were more numerous in the DA. However, activation of microglia was much more pronounced in the DA strain while it remained virtually the same in the PVGA strain. Thus, a kinetic analysis reveals a strong immune cell response in the DA strain characterized by a profound infiltration

of granulocytes and NK cells early after injury, which is followed by a strong activation of microglia. In contrast, the PVGA strain reveals a more moderate response in all aspects of the immune cell response (Fig. 10 B). At 18 days after TBI, the numbers of activated macrophages and microglia were decreased and though higher numbers were observed in the DA strain, this did not reach statistical significance. However, the percentage of T lymphocytes in DA was significantly higher than the PVGA at 18 DPI.

As the complement system was the top regulated pathway between the two strains, we looked further into it to characterize the differences and also the role of the complement system in our model. Using RT-PCR we found that the complement factors *C1q*, *C3* and *Itgam/CD11b* (complement receptor 3, CR3) were more expressed in the DA strain both in naïve state and at 1 and 6 DPI. These differences were increased with time from naïve -> 1DPI -> 6 DPI and the kinetics was similar to the kinetics of microglia activation in both strains (Fig. 10 B and C). This similarity was particularly observed for *C1q* and *C3* while *CD11b* displayed a decline in relation to the microglia kinetics. This can possibly be explained by the decline in numbers of macrophages at 6 DPI, since these cells also express *CD11b*. The exact cell origin of C3 production was not examined here. However, a previous study in the same TBI model utilizing *in situ* hybridization has shown that C3 is produced by microglia in the WDI model (Bellander et al. 1996). Furthermore, the similarities in C3 – microglia kinetics and the fact that we found more microglia cells that stained for C3 in the thalamus in the DA strain points towards a microglial production of C3 also in our experiments.

The complement system exerts multiple roles in CNS diseases with both healing and deleterious characteristics; reviewed in many articles (Elward & Gasque 2003; Francis et al. 2003; Morgan et al. 1997; Perry & O'Connor 2008; Veerhuis et al. 2011). Complement factor fragments (C1q, C3b, iC3b) can bind to receptors (CR3/CD11b) on apoptotic cells and thereby facilitate their phagocytosis and promote healing. On the other hand, they can produce the membrane attack complex (MAC) that attaches to the cell membranes to promote lysis, attract pro-inflammatory cells to the injured tissue (C5a) and promote synaptic stripping (C1q, C3). In our experiments, utilizing confocal microscopy, we provide evidence of both the tagging function of C3 and lytic function of MAC on neurons following TBI. Thus, we saw a C3 and MAC dot-like staining of neurons in the pericontusional cortex. This is in accordance with previous studies in the WDI model and human TBI showing C3d and C9 to stain neurons at the vicinity of the lesion (Bellander et al. 1996; Bellander et al. 2001). However, in our studies, C3 and MAC did not only stain neurons in the vicinity of the lesion but also stained neurons and neuronal axons in the hippocampus providing evidence of a more remote activity of C3 and MAC, possibly regulated by a local production of complement factors. This notion is supported by the observation that a diffuse C3 and MAC staining, that was present at 1 DPI and possibly originated from blood derived complement factors, was diminished at 6 DPI.

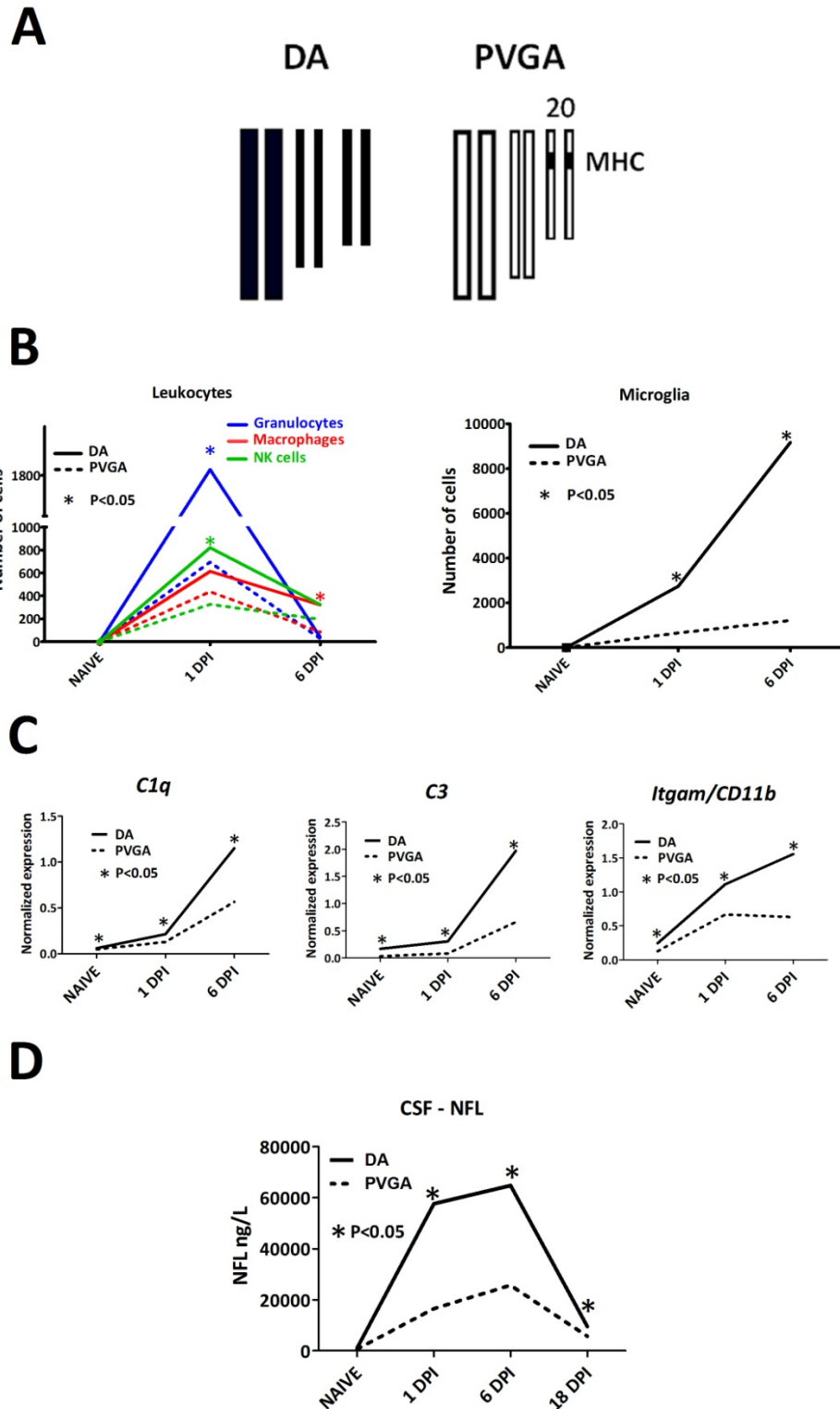


Figure 10. Strain regulation and kinetics of secondary injury cascades by *non-MHC* genes (A). B) Strain differences and kinetics in leukocyte infiltration and microglia activation at 1 and 6 DPI. C) Strain differences and kinetics of *C1q*, *C3* and *CD11b* expression. D) Strain differences and kinetics of NFL levels in rat CSF. The graphs in B-D were produced, using the mean numbers per strain and time-point from paper I, to illustrate the kinetics for each strain. The number of leukocytes and activated microglia in naïve animals are virtually zero (unpublished data from the lab). Numbers for individual rats are shown in the graphs in paper I. * on DA depicts statistically significant differences compared to PVGA at a certain time-point.

Neuronal/axonal injury and outcome was assessed by various methods: 1) Neurofilament light (NFL) level quantification in the CSF in paper I; 2) Lesion volume quantification in paper II; 3) Estimation of the numbers of surviving neurons in the hilus, by using design-based stereology, in paper III.

CSF NFL levels are increased in EAE (Norgren et al. 2005) and correlate to axonal damage and disease severity in MS (Norgren et al. 2004; Salzer et al. 2010; Teunissen et al. 2005). Also, CSF NFL levels correlate to the degree of disease progression in amyotrophic lateral sclerosis (Tortelli et al. 2012). The kinetics of CSF – NFL levels was the same in the two strains at the examined time points. However, we found that the DA strain displays considerably higher levels of NFL compared to PVG at all time-points and especially at 1 and 6 DPI after TBI (Fig.10 D). Thus, more severe neuronal/axonal injury was observed in the DA strain. This is in accordance with our findings that more neurons survive in the hilus of the PVGA rat at 30 DPI. Surprisingly, when we counted the lesion volumes at 18 DPI, we found that DA rats displayed smaller contusion volumes than PVGA rats. Although a high degree of variation was observed, particularly in the PVGA strain, it did reach statistical significance. This is thus contradicting the results of higher CSF-NFL levels and less neuronal number estimates in the DA rat. Two possibilities, apart from the difference in time-points and methodological aspects, may underlie this apparent contradictory finding. First, a possible higher rate of proliferation and activation of glia cells in the DA strain might have contributed to smaller lesion volumes. If this is true, it raises a concern about the validity of this method as a measure of outcome, especially when different animal strains are studied but also when treatment that affects the glial response after TBI is used. A second possible explanation is that cortical neurons differ from hippocampal neurons with regards to cell death mechanism and might be differentially regulated between the strains. This is supported by the findings of Wennersten et al that neurons in the lesion display a necrotic morphology, in the pericontusional cortex both a necrotic and apoptotic morphology and in the hippocampus only apoptotic morphology in the WDI model (Wennersten et al. 2003). It might thus be speculated, that a higher immune cell influx in the DA strain results in a more effective removal of necrotic cells from the cortex while more effective mechanisms in the PVGA strain save hippocampal neurons from apoptosis. Nevertheless, the combined finding of higher NFL levels early and a later augmented loss of hippocampal neurons strongly indicate that the DA response is associated with a deleterious outcome with regard to nerve cell survival both in the short and medium term after experimental TBI.

In summary, we have shown that *non-MHC* genes regulate secondary injury cascades, particularly neuroinflammation and neurodegeneration. Several questions arise from this; some of them have been explored to a certain extent and are addressed in the

following sections: 1) What is the role of *MHC*-genes (4.2); 2) What are the specific loci/genes and what do they regulate (4.2 and 4.3).

4.2 MHC GENES ACCOUNT FOR STRAIN DIFFERENCES IN MHC-II PRESENTATION WITH A SMALLER CONTRIBUTION FROM *CIITA* AT THE TRANSCRIPTION LEVEL

In paper I we found substantial differences in the numbers of microglia and monocytes/macrophages that present MHC-II on their surface at 6 DPI, and this was also true for microglia at 1 DPI. We also saw that the DA strain has higher levels of expression of the invariant chain *CD74* and *RT1B α* compared to PVGA (paper II). Thus, the quantitative differences in MHC-II molecules could likely be ascribed to a *non-MHC* regulation of their production at the transcription level. Studies in bare lymphocyte syndrome, a hereditary syndrome where cells lack the ability to produce MHC-II, and experiments on *Ciita*-deficient mice, have shown *Ciita* to be the master regulator of MHC-II expression (Chang et al. 1996; Steimle et al. 1993). In turn, expression of *Ciita* is highly complex and is *cis*-regulated involving four different promoters (Muhlethaler-Mottet et al. 1997). In paper II, we hypothesized that the *non-MHC* gene that regulated MHC-II presentation between the DA and PVGA strains was *Ciita*. Indeed, using two reciprocal congenic strains containing the PVG *Ciita* haplotype on DA background and the DA *Ciita* haplotype on PVGA background, we found that variation in the *Ciita* haplotype regulates MHC-II expression with a subsequently similar quantitative effect on MHC-II presentation (Fig. 11). This is in accordance with previous findings in the VRA model that show *Ciita* to be the main regulator of MHC-II presentation (Lidman et al. 2003).

Antigen presentation by MHC class II molecules in the CNS has been studied mainly in MS and its animal model EAE in order to disclose mechanisms of autoimmunity. The HLA exerts the by far strongest genetic effect on disease susceptibility, which is also supported by rat studies in EAE (Gourraud et al. 2012; Weissert et al. 1998). However, there are now indications that the HLA might also affect primarily non-autoimmune diseases. *MHC* genes affect neuropathic pain in an experimental model of nerve injury and some studies have shown a possible association of single-nucleotide polymorphisms (SNPs) located within HLA to Parkinson's and Alzheimer's disease (Ahmed et al. 2012; Dominguez et al. 2008; Guerini et al. 2009). In paper II we studied the effect of *MHC*-gene variation in TBI. This was done by using the PVG rat, which is homozygous for the *c* alleles (*RT1^c*) and the congenic PVGA that is homozygous for the *av1* (*RT1^{av1}*). We first studied the expression of the class II invariant chain (*Cd74*) and the α chain of *RT1B* and found no regulation by *MHC* genes. Surprisingly, when we stained for RT1B (OX6) we found major differences with more RT1B staining on PVG rats with the RT1^{av1} haplotype and this finding was replicated using flow cytometry.

Thus, we found that *MHC* genes account for quantitative differences in MHC-II presentation after TBI at the protein level. The highly polymorphic nature of *MHC*-genes (see 1.1.4) and the complexity of the MHC-II antigen presentation pathway make it difficult to identify the specific gene that accounts for the strain differences and also to find exactly where in the pathway it exerts its effect. The MHC-II presentation pathway is shown in figure 11: Newly synthesized MHC II $\alpha\beta$ dimers assemble in with the chaperone invariant chain (Ii). Ii inserts its class II-associated Ii peptide (CLIP) within the peptide-binding site of the dimer in order to stabilize and protect the site from interacting with other peptides. The $\alpha\beta$ -CLIP complexes are transported towards sites of antigen binding where the phagocytosed and processed antigen displaces CLIP and enters the antigen binding peptide. The $\alpha\beta$ -peptides are then transported towards the membrane for MHC-II presentation or towards the lysosomes for degradation. MHC-II surface molecules can also be internalized and transported for degradation. Thus, the amount of MHC-II surface presented molecules depends on the whole turnover including the degradation phase and not only on the production of $\alpha\beta$ -peptide complexes (Fig. 11) (Castellino et al. 1997; Villadangos et al. 2005).

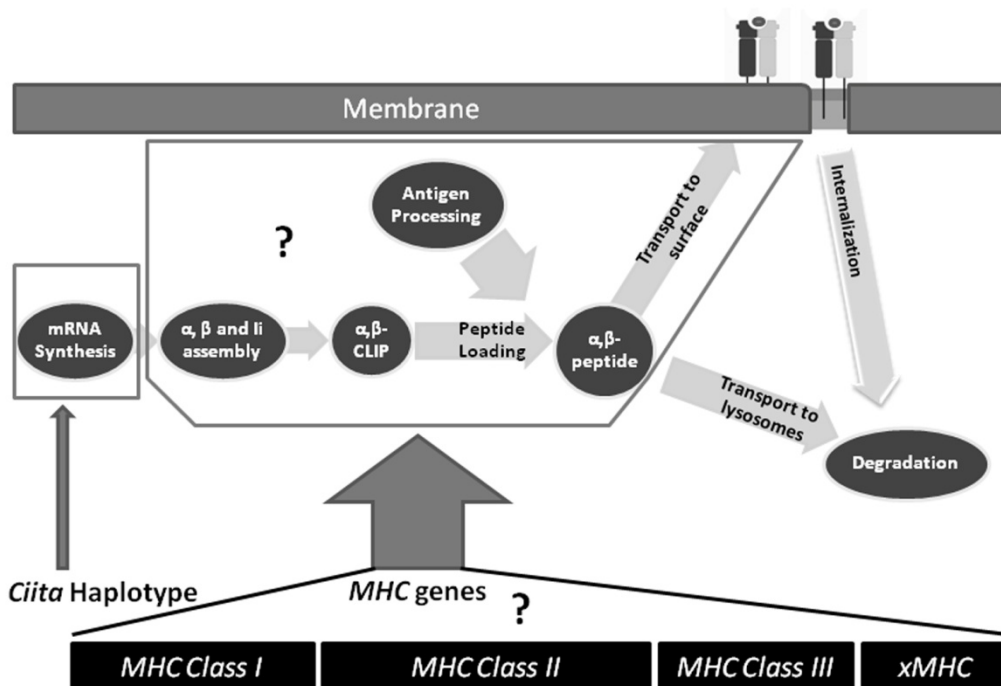


Figure 11. The major histocompatibility complex class II (MHC II) antigen-presentation pathway regulated by the *Ciita* and *MHC*-complex after TBI; as discussed in the text.

We did not find differences between the $RT1^C$ and $RT1^{Av1}$ haplotypes in *RT1Ba* and *Cd74* expression, thereby excluding an effect at the level of transcription. Also, we did not find any overt differences in terms of microglia/monocyte numbers and activation (as shown by *CD11b* and *Mrf1* expression analysis and $CD11b^+$ cells), astroglial

response (as shown by *Gfap* expression analysis), TNF production (as shown by *Tnf* expression analysis), *Ager*/RAGE formation and RT1D presentation (OX17 immunohistochemistry, unpublished results). Furthermore, flow cytometry matched the results of immunohistochemistry and since it was performed on non permeabilized cells, it should imply that the differences in MHC-II numbers concern surface presented and not intracellular/internalized MHC-II, possibly excluding a genetic effect on the degradation phase. Altogether, these findings suggest that the regulatory effect is induced by the *class II* region, possibly due to RT1B-peptide binding affinity. This is also supported by the finding that genetic variations between the *av1* and *c* haplotype exert only a small effect on MHC-II presentation in rat VRA. A possible explanation for this contradiction could thus be that the inherent differences between the models result in a diversity of the peptides that are presented on MHC-II molecules in the VRA and TBI models, respectively. However, it has to be emphasized that these conclusions are based only on indirect evidence and that intra-MHC congenic rats should be used in experimental TBI to help us disclose the gene(s) and mechanisms that account for the differences.

The differences in MHC-II presentation found between the PVG and PVGA strains can be utilized to investigate if autoimmune responses occur after TBI. CD4⁺ lymphocytes can react with MHC-II presented peptides and become activated. This is dependent on the degree of APC maturation and the presence of co-stimulatory molecules (Aloisi et al. 2000). Microglia are considered to be the professional APCs of the brain while the role of astrocytes is controversial (Aloisi et al. 1998; Chastain et al. 2011; Cornet et al. 2000; Stuve et al. 2002). MHC-II presentation in our model occurs on CD11b⁺ cells, microglia or macrophages, and these cells also express the co-stimulatory molecule B7.2, thereby increasing the likelihood for an autoimmune response (Fig. 12).

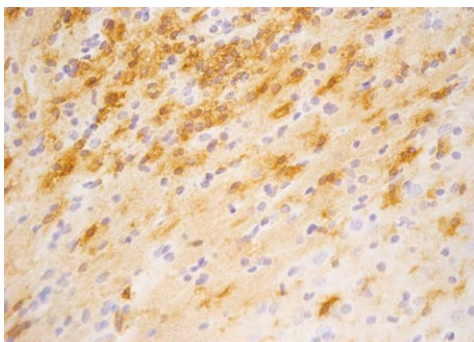


Figure 12. Immunohistochemical staining showing the presence of B7-2 at the perilesional cortex after TBI

We found more T cells in the PVGA strain compared to PVG at 18 DPI which suggests a stronger T cell response for the *av1* haplotype. This is in accordance with the larger number of MHC-II⁺ cells present in this strain. However, we cannot exclude that the peptides presented by the *av1* and *c* MHC-II molecules differ and result in stronger affinities to CD4⁺ cells thereby affecting their activation status. Higher numbers of T

cells could thus account for a stronger autoimmune response in the PVGA strain. In an attempt to disclose an encephalitogenic response in our model we also stimulated lymphocytes from cervical lymph nodes with a myelin basic protein derived peptide, MBP₆₃₋₈₈. This particular peptide was chosen since it has been shown to induce EAE on Lewis rats with the *av1* or *c* haplotype, showing that it can bind to MHC-II molecules of both haplotypes and thereby elicit an autoimmune response in both the PVG and PVGA strains (de Graaf et al. 1999). Recall with MBP₆₃₋₈₈ did not give any evidence of autoimmunity directed against this peptide. However, this does not exclude autoimmunity directed against other peptides, since there is a large repertoire of peptides that can elicit an encephalitogenic response in the context of CNS injury (Ankeny & Popovich 2009; Fissolo et al. 2009). Although no differences in lesion volume could be ascribed to *MHC* genes, this does not rule out differences in other types of outcome measurements such as behavioral and memory tests.

4.3 NATURALLY OCCURRING VARIATION IN THE *GSTA4* GENE REGULATES NEURODEGENERATION

In paper I, we found the glutathione metabolism pathway to be the most regulated transcriptional pathway between the DA and PVGA strains after TBI. At the same time we conducted experiments in a rat VRA model to find gene variations that regulate neurodegeneration after nerve axotomy. These experiments were based on previous work in our laboratory that started some years ago and demonstrated a higher degree of neurodegeneration in the DA strain compared to PVGA, with 26% and 50% motorneuron survival, respectively for each strain (Lundberg et al. 2001). A subsequent study in a DA x PVG intercross pinpointed a locus at chromosome 8 (VRA1) to regulate neurodegeneration (Lidman et al. 2003). Following from this finding, a genetic fragment that corresponds to the *Vra1* locus was introgressed from the PVGA strain into DA background to produce a congenic strain (DA.PVG^{*av1*}-*Vra1*-R3) that was indeed shown to display higher motorneuron survival compared to the PVGA strain (Swanberg et al. 2009). In paper III, a congenic that narrowed down the introgressed PVG fragment to 35 genes (DA.PVG^{*av1*}-*Vra1*-R5), still displayed better motorneuron survival than the parental DA. Starting from this finding and in parallel with our TBI experiments, we tried to find candidate genes residing in the *Vra1* locus that could regulate motorneuron degeneration. Given that it is very difficult to produce one-gene congenics we performed an eQTL mapping in an F2 DA x PVGA intercross. The L4 segment was dissected 5 days after VRA and analyzed by Affymetrix Exon array for expression profiling of more than 27.000 genes. eQTL mapping revealed only one strong *cis*-regulated gene contained in the R5 fragment, *Gsta4*. *Gsta4* was also one of the four genes that were included in the glutathione metabolism pathway and were differentially regulated between the DA and PVGA strain after TBI. When we performed WDI in DA, PVGA and R5 rats, we found that the estimated number of

surviving neurons in the hilus at 30 DPI was higher for the PVGA and R5 strains compared to the DA strain. Thus, our studies in VRA and TBI pointed towards *Gsta4* to regulate neurodegeneration in both models. Gene expression and western blot analysis showed that R5 displays higher mRNA and protein levels compared to DA in both strains, suggesting that it is the quantity of *Gsta4* that regulates the neurodegenerative effect. Immunohistochemical investigations showed *Gsta4* to be present mainly in neurons in the spinal cord and in the brain, particularly in the hippocampus.

Gsta4 displays by far the highest capability for detoxifying 4-HNE by catalyzing its conjugation to GSH, suggesting that this might be the pathway by which higher levels of *Gsta4* in neuronal cells lead to a more efficient detoxification of 4-HNE and thereby reduced neurodegeneration. This is supported by previous findings that 4-HNE increases cell death in neurons, both in vitro and in vivo (Abarikwu et al. 2012; Akude et al. 2010; McCracken et al. 2000; Vigh et al. 2005). In our WDI model, we found 4-HNE immunoreactivity both in the pericontusional area and the hippocampus, including neurons, in accordance with previous studies in experimental TBI and suggesting that it may contribute to the DA/R5 strain differences in neuronal cell loss. This was further corroborated by injecting 4-HNE in the brain of DA and R5 rats, where it induced apoptosis in neuronal cells with higher levels of NFL in the DA compared to R5. Altogether these results suggest that *Gsta4* variation is regulating neurodegeneration in experimental TBI.

The toxic activity of 4-HNE has been implicated to induce neuronal cell death in other neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's and Alzheimer's disease (Butterfield et al. 2010; Fukuda et al. 2009; Pedersen et al. 1998; Perluigi et al. 2005; Sultana et al. 2012; Yoritaka et al. 1996) and detection of 4-HNE in the CSF of patients with these disorders has been previously reported (Selley 1998; Selley et al. 2002; Simpson et al. 2004). In paper IV, we detected 4-HNE-adducts to be present in human pericontusional tissue, showing that the *Gsta4* – 4-HNE pathway might also be of relevance in human TBI. We then put a large effort to detect 4-HNE in CSF of experimentally contused rats and in the CSF of TBI patients and controls. The protocols have also included CSF from MS and amyotrophic lateral sclerosis ALS as references. First, a commercial ELISA kit was used, which successfully detected 4-HNE-his complexes in low levels in controls and elevated in TBI as well as MS. However, a large run on MS CSF revealed a conspicuous correlation to immunoglobulin index. This led to extensive control experiments where we later found that all signal was due to unspecific binding to immunoglobulins, and none specific for HNE. This finding is of major importance for already published studies where detection of HNE was done with the same ELISA kit. A second commercial kit, with a sensitivity of 0,625 ng/ml, was also unable to detect a positive signal in the samples. To verify these findings, we set up our own Sandwich Elisa with two antibodies: monoclonal mouse anti-HNE as

capture antibody and rabbit polyclonal anti HNE as detection antibody. One hundred μL CSF was incubated overnight and detection antibody was applied in the same amount (1:5000), also overnight. Signal enhancement with avidin-biotin system was applied to increase sensitivity. As standard, we used control CSF, incubated with purified HNE (Cayman chemicals) and diluted into different concentrations. This approach could verify a reliable signal down to 0,5 μM added HNE with a good consistency between triplicates, but still no positive signal in any sample, neither control nor disease. Incubated CSF was also run with western blot using the above mentioned antibodies and both showed a nice spectrum of protein bands in CSF incubated with 4-HNE, but failed to identify any 4-HNE positive bands in TBI/MS/Control CSF. The CSF used has been carefully taken and immediately centrifuged followed by storage at -80°C . Based on these extensive experiments, we therefore concluded that it is unlikely that 4-HNE exist in micromolar concentrations as previously reported. Perhaps, 4-HNE modified proteins are present at a nano- or picomolar concentration, if analyzed by more sensitive methods such as HPLC. Alternatively, microdialysis fluid should also be analyzed for 4-HNE, since markers of oxidative stress such as 8-iso-PGF2a have been shown to display higher levels in microdialysis fluid compared to CSF (Clausen et al. 2012).

Altogether, our findings reveal a natural variation in *Gsta4* to regulate neurodegeneration in both VRA and experimental TBI and identify the *Gsta4* – 4-HNE pathway as an interesting candidate for regulating outcome in human TBI.

5 POINTS OF PERSPECTIVE

In summary, this thesis highlights a substantial impact of genetic heterogeneity on secondary pathophysiological mechanisms that follow brain trauma and identifies specific loci/genes that regulate specific pathways. Below, I try to summarize the most essential results and give my personal thoughts as to their relation to current knowledge in the field and also to future experiments required to advance the findings of this thesis.

- **FORWARD GENETICS IN EXPERIMENTAL TBI**

Forward genetics have been mainly used in experimental models of complex diseases e.g. rheumatoid arthritis, diabetes and MS, among many others. In complex diseases, multiple genes regulate the disease and often these genes exert only a small effect to increase the overall disease susceptibility in interplay with environmental factors (Pritchard & Cox 2002; Ramos & Olden 2008). In TBI the environmental factor which causes the disease, although heterogeneous, is known: head trauma. Most of the TBI research has focused on observational or hypothesis-driven mechanistic studies to elucidate the secondary cascades that connect the type of head trauma to the outcome. Although this has contributed a lot to the understanding of TBI pathology, our knowledge is still insufficient. In recent years, results from association studies in human TBI suggest also a role for genetic factors, although to what extent is largely unknown. This was tested experimentally in this thesis and revealed a substantial impact of genetic heterogeneity on TBI pathways and outcome in the rat. With the use of forward genetics we identified *non-MHC* genes to regulate inflammation and neuronal/axonal injury, *Gsta4* to be a candidate gene that regulates hippocampal neuronal loss and *MHC*-genes and *Ciita* to regulate MHC-II presentation after TBI. Thus, we identified genetic loci and genes which exert genetic effects in pathways that are specific for each locus/gene. We can thus confidently say that TBI is a “complex” disorder with a substantial contribution of allelic heterogeneities on disease pathways and outcome. In this context, some of the findings of this thesis are illustrated in figure 13. It should be emphasized that each gene’s and locus contributions could be independent of the other or it can be multiplicative as a result of gene-gene interactions or even protein-protein interactions (Plomin et al. 2009). Also, even though we find significant regulation by certain genes/loci, we do not know if these are the genes with the strongest effect. For example data from the Affymetrix analyses showed that the DA strain has a limited capability to produce lipocalin-2 (*Lcn2*) compared to PVGA and this has been verified in ongoing studies. *Lcn2* has been shown to modulate the phenotype of microglia and astrocytes and has detrimental

effects in experimental spinal cord injury (Lee et al. 2007; Lee et al. 2009; Rathore et al. 2011). It thus can be of significance in regulating the glia response between the two strains. In addition, a large number of other transcripts were differentially regulated after injury between the strains. Furthermore, the genes and loci identified here to regulate response to TBI might or might not be the same when other strains are utilized. For all the above reasons, further studies utilizing the forward genetics method in other rat strains and in crossing experiments are therefore encouraged.

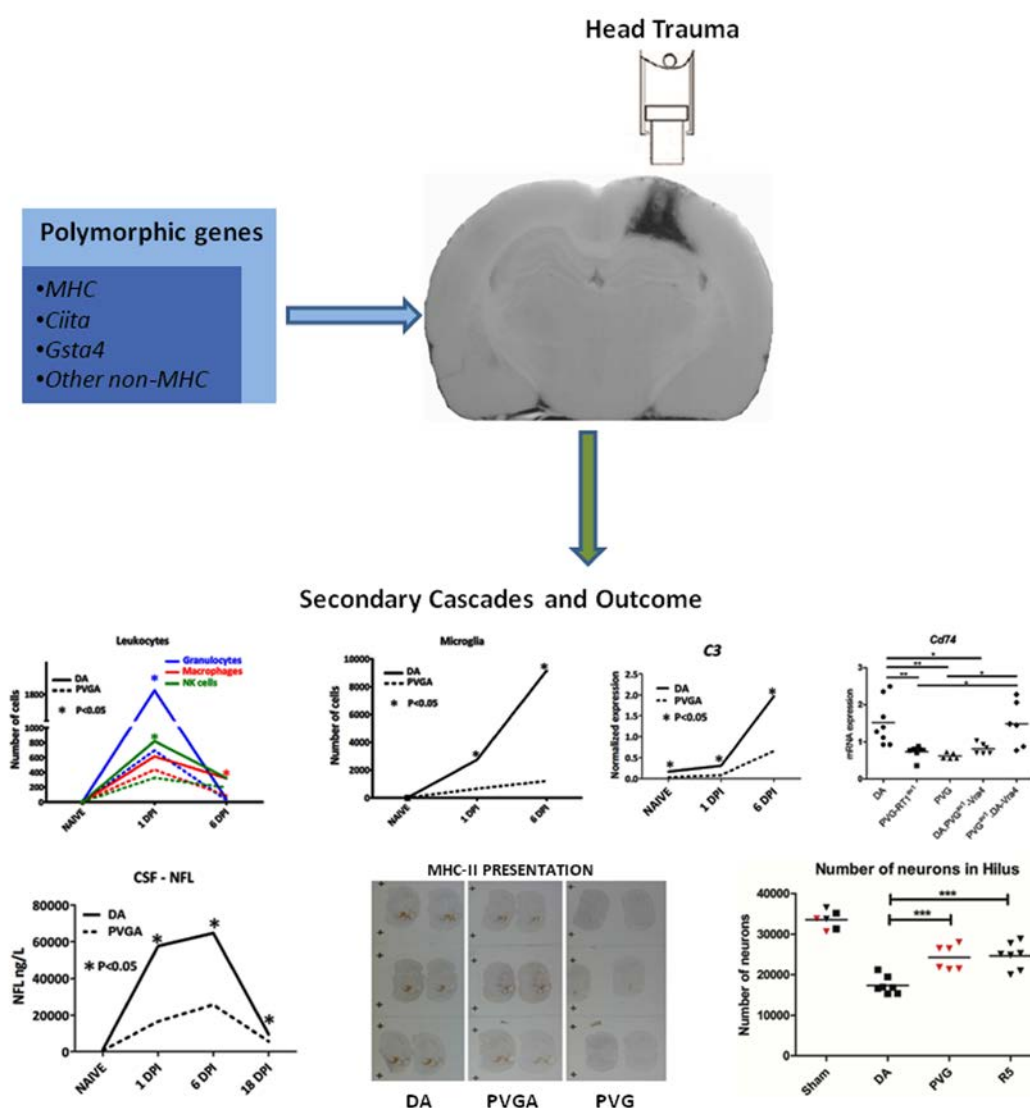


Figure 13. Forward genetics were used to dissect the influence of inherent genetic variation on secondary cascades and outcome. Inbred rat strains and congenics were studied in experimental TBI. The experiments were performed with identical injury parameters for all strains and revealed multiple genes/loci that influence specific pathways and outcome.

The results from this thesis together with previous studies (Bellander et al. 2010; Reid et al. 2010; Tan et al. 2009; Witgen et al. 2006), prove genetic heterogeneity to be one of the possible explanations for some of the contradicting results in experimental TBI

investigations. In addition, genetic heterogeneity can possibly regulate response to treatment. Hypothetically, one could say that the DA strain could benefit more from a pharmacological treatment that targets inflammation or lipid peroxidation than the PVG strain, where the same treatment might not lead to a significant improvement. If this proves to be the case, it will be of importance to conduct future studies on pharmacological agents in both inbred and outbred strains. This should be done in order to rule out or identify genetic factors that might regulate response to treatment.

- **NEURODEGENERATION OR NEUROINFLAMMATION**

In paper IV, we found that *Gsta4* is a candidate gene to regulate 4-HNE mediated apoptosis and also neuronal cell death in the hilus. Recent technological advancements that enable the production of transgenic rats can now be used to investigate if *Gsta4* is really the gene and further study its function and effect in TBI. We are therefore, in an ongoing study, generating a *Gsta4* over-expressing DA rat to be tested in the same WDI model. Also, since the toxic activity of 4-HNE has been implicated to induce neuronal cell death in other neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's and Alzheimer's disease, studies of the DA, R5 and *Gsta4* over-expressing DA rat in models of these diseases are relevant (Butterfield et al. 2010; Fukuda et al. 2009; Pedersen et al. 1998; Perluigi et al. 2005; Sultana et al. 2012; Yoritaka et al. 1996). Furthermore, our results encourage investigations on therapeutic agents, such as fumaric acid esters, which are emerging now as possible treatments in MS and can increase *Gsta4* expression, via the nuclear factor (erythroid-derived 2)-like 2 (*nrf2*) pathway, also in TBI (Linker et al. 2011; Porritt et al. 2012). In addition, pharmacological agents, such as histidine analogues or hydrazine derivatives, which scavenge 4-HNE and rescue cells from 4-HNE toxicity, might also have an effect in TBI (Galvani et al. 2008; Hall et al. 2010; Tang et al. 2007). A major part of the toxic activity of 4-HNE is due to its binding to proteins that alters their activity and function. In this way, 4-HNE can modify proteins to produce neoantigens and facilitate epitope spreading, a concept that is particularly interesting in the context of autoimmune diseases (Kurien & Scofield 2008). This role of 4-HNE has been studied in rheumatologic diseases, while modification of MOG by another aldehyde, MDA, led to a more aggressive EAE disease (Scofield et al. 2005; Wallberg et al. 2007). Studies on the role of *Gsta4* variability and 4-HNE in EAE and MS are therefore also encouraged from an immunological point of view.

In paper I, we find that the DA strain displayed stronger activation of inflammatory pathways and also more neurodegeneration compared to PVG. The detrimental effects of dysregulated activation of microglia, macrophages, neutrophils and the complement system have been previously demonstrated in TBI and can explain the

above correlational connection between neurodegeneration and inflammation. Furthermore, the finding of an influx of two different subsets of NK cells, differing between the rat strains, encourages further studies on the role of NK cells in TBI. These subsets have been ascribed different functions with regards to T cell activation and cytotoxicity: CD161 bright NK cells show a lower inhibitory activity towards T cell activation and a higher cytotoxic activity compared to the CD161 dim NK cells (Inngjerdingen et al. 2012; Kheradmand et al. 2008). Based on this finding and on recent research, revealing different phenotypes for activated microglia and macrophages, one should explore if also qualitative differences in these cells account for differences in the inflammatory response between the DA and PVG strain (Martinez et al. 2008; Ransohoff & Perry 2009; Saijo & Glass 2011).

In the context of genetic regulation we are not able to confidently say if genetic variability acting on neurodegenerative pathways (such as *Gsta4*) accounts for the differences in inflammatory response after TBI (paper IV) or, vice versa, that genetic regulation of primarily inflammatory factors accounts for the differences in neurodegeneration between the strains (paper I). However, based on our findings and on previous studies in VRA and EAE on these strains, the most plausible explanation is that genetics regulate both inflammatory and neurodegenerative mediators, which then interact at the gene, protein and cellular level to drive the disease phenotype.

- **THE ROLE OF MHC-II PRESENTATION: TBI AND AUTOIMMUNITY**

In paper II, we found a quantitative genetic regulation of MHC-II presentation mainly by *MHC* genes, but also by *Ciita*. Furthermore, T cell infiltration was regulated by *MHC* genes. This should be further explored as to the significance of these findings with regard to outcome. MHC-II presentation can lead to activation, anergy or apoptosis of CD4⁺ T cells. This is largely unexplored in the field of CNS trauma as it is generally considered that traumatic brain or spinal cord injuries (SCIs) do not lead to autoimmunity. However, there are several indications that autoreactive T and B cells are generated after CNS injuries. “Signs of autoimmunity” have been shown to occur after SCI, TBI and also stroke (Ankeny & Popovich 2009; Ankeny & Popovich 2010; Becker et al. 2011; Cox et al. 2006). One study showed that T cells obtained from patients with TBI can react towards myelin as a sign of autoimmunity (Cox et al. 2006). In addition, some reports have shown the presence of autoantibodies in the sera of patients with TBI providing indirect evidence about the function of lymphocytes (Lopez-Escribano et al. 2002; Prochazka et al. 1971; Skoda et al. 2006). We also, in a previous study, found autoantibodies in the serum of experimentally contused rats (Rudehill et al. 2006). Similarly the existence of autoreactive T cells and production of autoantibodies have been demonstrated in patients with SCI (Davies et al. 2007;

Hayes et al. 2002). The group of Popovich has also demonstrated a destructive role of autoreactive T and B cells in experimental SCI where the location of injury and the exact timing when activation of these cells is studied play a substantial role (Ankeny et al. 2009; Lucin et al. 2007; Popovich et al. 1996). However, animals subjected to traumatic CNS injuries do not spontaneously develop clinical signs of autoimmunity. This could be because regulatory mechanisms suppress the activity of autoimmune cells (Ankeny & Popovich 2010; Kipnis et al. 2002). These autoregulatory mechanisms could be surpassed when a new “foreign” antigen is introduced so that autoimmunity might occur. When this is done by immunization where adjuvant is used it has been shown to have beneficial effects (Schwartz & Kipnis 2001). It is still unclear what the result would be if a new antigen is introduced if this happens in an unprimed setting. Human studies have been contradicting as to whether TBI increases the risk for MS (Goldacre et al. 2006; Kang & Lin 2012; Pflieger et al. 2009; Poser 1994).

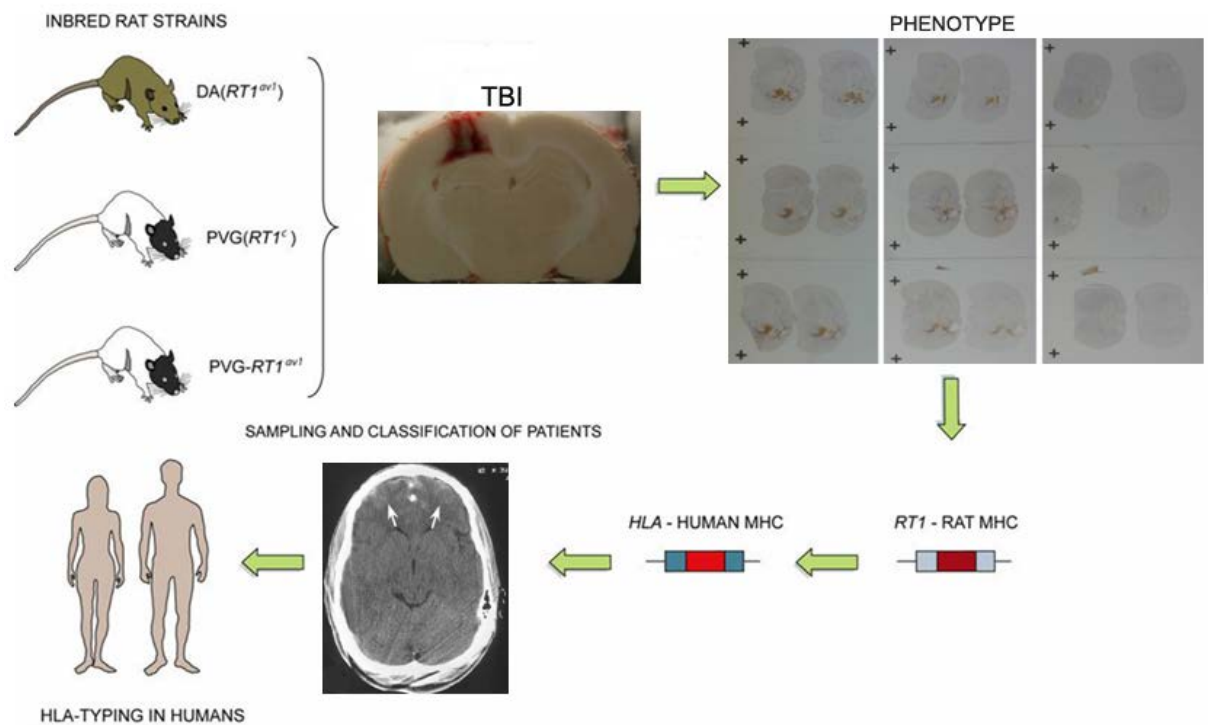


Figure 14. Example of a translational approach from experimental TBI (paper II) to human TBI. We find MHC-II presentation after TBI to be regulated by *MHC* genes. This could also be investigated in human TBI by genotyping in order to explore if HLA polymorphisms influence disease outcome (e.g. functional restitution, biomarkers reflecting inflammation).

The reasons for this contradiction have been speculated to be the variation in study designs, the different ethnicities studied and also differences in local environmental factors triggering the disease at a later time point after TBI. One explanation could also be that quantitative and/or qualitative differences in MHC-II presented antigens, depending on both the genotype (HLA) as shown in our study and also the triggering

antigen, which occur after TBI, influence a later re-activation of autoimmunity and risk for MS. This is particularly interesting since new data show that the location of initial antigen presentation or epitope spreading in MS might occur in the CNS itself and not in the periphery (Finsen & Owens 2011; Sosa & Forsthuber 2011). It is thus plausible that future studies that investigate the relation of TBI to MS should also explore if a certain haplotype in combination with TBI increases the risk for MS in a subpopulation of patients (Fig. 14).

• **TRANSLATIONAL INTERPRETATION**

The ultimate goal of investigations performed in experimental TBI is to provide information that will increase the understanding of the pathophysiology of the disease in humans and lead to successful treatment.

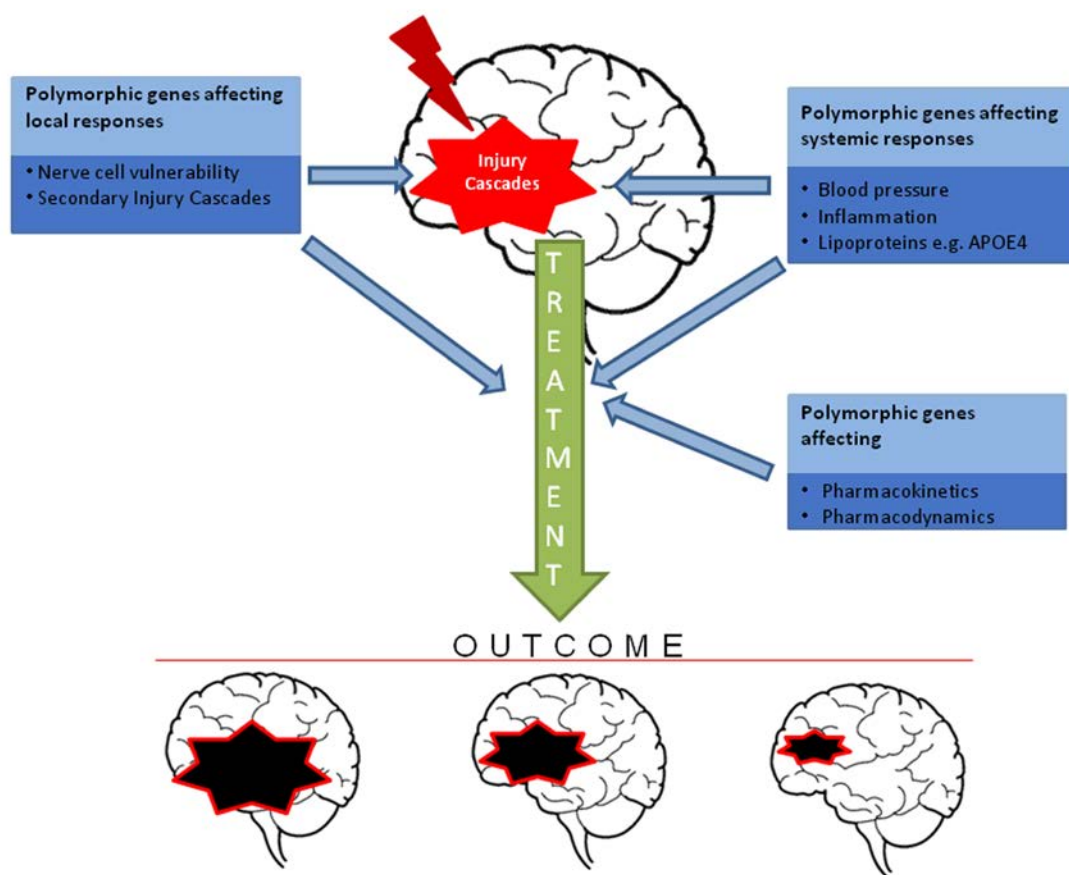


Figure 15. Hypothetical model explaining why similar individuals with the same type of injury display a different outcome also in treatment studies.

It is thus of major importance that the findings of this thesis are further investigated in human TBI. In the previous section an example of a future translational study to test for the effect of MHC genes in human brain trauma is introduced. Similarly, the role of

Gsta4 polymorphism in regulating neurodegeneration as well as the role of 4-HNE as a possible disease biomarker should be investigated in human TBI. For the former purpose an association study will reveal if *Gsta4* is relevant in the human context while for the latter purpose the use of more sensitive methods such as HPLC and/or analyses in microdialysis fluid instead of CSF might be utilized.

As an overall conclusion, the findings of this thesis show that there is a substantial contribution of genetic heterogeneity on disease pathways and outcome after experimental TBI. This, when translated to the human context, can explain why similar individuals with the same type of injury present different outcomes after TBI and might also affect response to treatment (Fig. 15). For example, it cannot be excluded that treatment with corticosteroids, shown to have detrimental effects in the CRASH study, can have beneficial effects for a subgroup of TBI patients with a genetically regulated more vigorous or dysregulated inflammatory response (Edwards et al. 2005; Roberts et al. 2004). This underscores the need for a GWAS that can reveal genetic polymorphisms relevant for human TBI outcome and also for finding disease biomarkers that reflect activation of specific pathways, in turn of importance for developing more efficient individualized therapies.

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7 REFERENCES

- Abarikwu, S. O., Pant, A. B. & Farombi, E. O. (2012). 4-Hydroxynonenal induces mitochondrial-mediated apoptosis and oxidative stress in SH-SY5Y human neuronal cells. *Basic Clin Pharmacol Toxicol*, 110 (5): 441-8.
- Adibhatla, R. M. & Hatcher, J. F. (2010). Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal*, 12 (1): 125-69.
- Ahmed, I., Tamouza, R., Delord, M., Krishnamoorthy, R., Tzourio, C., Mulot, C., Nacfer, M., Lambert, J. C., Beaune, P., Laurent-Puig, P., et al. (2012). Association between Parkinson's disease and the HLA-DRB1 locus. *Mov Disord*, 27 (9): 1104-10.
- Akude, E., Zherebitskaya, E., Roy Chowdhury, S. K., Girling, K. & Fernyhough, P. (2010). 4-Hydroxy-2-nonenal induces mitochondrial dysfunction and aberrant axonal outgrowth in adult sensory neurons that mimics features of diabetic neuropathy. *Neurotox Res*, 17 (1): 28-38.
- Aloisi, F., Ria, F., Penna, G. & Adorini, L. (1998). Microglia are more efficient than astrocytes in antigen processing and in Th1 but not Th2 cell activation. *J Immunol*, 160 (10): 4671-80.
- Aloisi, F., Ria, F. & Adorini, L. (2000). Regulation of T-cell responses by CNS antigen-presenting cells: different roles for microglia and astrocytes. *Immunol Today*, 21 (3): 141-7.
- Ankeny, D. P., Guan, Z. & Popovich, P. G. (2009). B cells produce pathogenic antibodies and impair recovery after spinal cord injury in mice. *J Clin Invest*, 119 (10): 2990-9.
- Ankeny, D. P. & Popovich, P. G. (2009). Mechanisms and implications of adaptive immune responses after traumatic spinal cord injury. *Neuroscience*, 158 (3): 1112-21.
- Ankeny, D. P. & Popovich, P. G. (2010). B cells and autoantibodies: complex roles in CNS injury. *Trends Immunol*, 31 (9): 332-8.
- Ansari, M. A., Roberts, K. N. & Scheff, S. W. (2008). A time course of contusion-induced oxidative stress and synaptic proteins in cortex in a rat model of TBI. *J Neurotrauma*, 25 (5): 513-26.
- Ariza, M., Matarin, M. D., Junque, C., Mataro, M., Clemente, I., Moral, P., Antonia Poca, M., Garnacho, A. & Sahuquillo, J. (2006). Influence of Angiotensin-converting enzyme polymorphism on neuropsychological subacute performance in moderate and severe traumatic brain injury. *J Neuropsychiatry Clin Neurosci*, 18 (1): 39-44.
- Awasthi, Y. C., Yang, Y., Tiwari, N. K., Patrick, B., Sharma, A., Li, J. & Awasthi, S. (2004). Regulation of 4-hydroxynonenal-mediated signaling by glutathione S-transferases. *Free Radic Biol Med*, 37 (5): 607-19.
- Bains, M. & Hall, E. D. (2012). Antioxidant therapies in traumatic brain and spinal cord injury. *Biochim Biophys Acta*, 1822 (5): 675-84.
- Balogh, L. M. & Atkins, W. M. (2011). Interactions of glutathione transferases with 4-hydroxynonenal. *Drug Metab Rev*, 43 (2): 165-78.
- Baskaya, M. K., Dogan, A., Temiz, C. & Dempsey, R. J. (2000). Application of 2,3,5-triphenyltetrazolium chloride staining to evaluate injury volume after controlled cortical impact brain injury: role of brain edema in evolution of injury volume. *J Neurotrauma*, 17 (1): 93-9.
- Bayir, H., Kagan, V. E., Tyurina, Y. Y., Tyurin, V., Ruppel, R. A., Adelson, P. D., Graham, S. H., Janesko, K., Clark, R. S. & Kochanek, P. M. (2002). Assessment of antioxidant reserves and oxidative stress in cerebrospinal fluid after severe traumatic brain injury in infants and children. *Pediatr Res*, 51 (5): 571-8.
- Bayir, H., Marion, D. W., Puccio, A. M., Wisniewski, S. R., Janesko, K. L., Clark, R. S. & Kochanek, P. M. (2004). Marked gender effect on lipid peroxidation after severe traumatic brain injury in adult patients. *J Neurotrauma*, 21 (1): 1-8.
- Bayir, H., Adelson, P. D., Wisniewski, S. R., Shore, P., Lai, Y., Brown, D., Janesko-Feldman, K. L., Kagan, V. E. & Kochanek, P. M. (2009). Therapeutic hypothermia preserves antioxidant defenses after severe traumatic brain injury in infants and children. *Crit Care Med*, 37 (2): 689-95.
- Beck, K. D., Nguyen, H. X., Galvan, M. D., Salazar, D. L., Woodruff, T. M. & Anderson, A. J. (2010). Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment. *Brain*, 133 (Pt 2): 433-47.
- Becker, K. J., Kalil, A. J., Tanzi, P., Zierath, D. K., Savos, A. V., Gee, J. M., Hadwin, J., Carter, K. T., Shibata, D. & Cain, K. C. (2011). Autoimmune responses to the brain after stroke are associated with worse outcome. *Stroke*, 42 (10): 2763-9.

- Bell, M. D., Taub, D. D. & Perry, V. H. (1996). Overriding the brain's intrinsic resistance to leukocyte recruitment with intraparenchymal injections of recombinant chemokines. *Neuroscience*, 74 (1): 283-92.
- Bellander, B.-M., Holst, H. v., Fredman, P. & Svensson, M. (1996). Activation of the complement cascade and increase of clusterin in the brain following a cortical contusion in the adult rat. *Journal of Neurosurgery*, 85 (3): 468-475.
- Bellander, B. M., Singhrao, S. K., Ohlsson, M., Mattsson, P. & Svensson, M. (2001). Complement activation in the human brain after traumatic head injury. *J Neurotrauma*, 18 (12): 1295-311.
- Bellander, B. M., Lidman, O., Ohlsson, M., Meijer, B., Piehl, F. & Svensson, M. (2010). Genetic regulation of microglia activation, complement expression, and neurodegeneration in a rat model of traumatic brain injury. *Exp Brain Res*, 205 (1): 103-14.
- Bellander, B. M., Olafsson, I. H., Ghatan, P. H., Bro Skejo, H. P., Hansson, L. O., Wanecek, M. & Svensson, M. A. (2011). Secondary insults following traumatic brain injury enhance complement activation in the human brain and release of the tissue damage marker S100B. *Acta Neurochir (Wien)*, 153 (1): 90-100.
- Birdsall Abrams, M., Josephson, A., Dominguez, C., Oberg, J., Diez, M., Spenger, C., Olson, L., Piehl, F. & Lidman, O. (2007). Recovery from spinal cord injury differs between rat strains in a major histocompatibility complex-independent manner. *Eur J Neurosci*, 26 (5): 1118-27.
- Bjugn, R. & Gundersen, H. J. (1993). Estimate of the total number of neurons and glial and endothelial cells in the rat spinal cord by means of the optical disector. *J Comp Neurol*, 328 (3): 406-14.
- Bochicchio, M., Latronico, N., Zani, D. G., Mariotti, M., Morandini, L., Acquarolo, A. M. & Candiani, A. (1990). Free radical-induced lipoperoxidation and severe head injury. A clinical study. *Intensive Care Med*, 16 (7): 444-7.
- Boyce, R. W., Dorph-Petersen, K.-A., Lyck, L. & Gundersen, H. J. G. (2010). Design-based Stereology. *Toxicologic Pathology*, 38 (7): 1011-1025.
- Brambilla, R., Bracchi-Ricard, V., Hu, W.-H., Frydel, B., Bramwell, A., Karmally, S., Green, E. J. & Bethea, J. R. (2005). Inhibition of astroglial nuclear factor κ B reduces inflammation and improves functional recovery after spinal cord injury. *The Journal of Experimental Medicine*, 202 (1): 145-156.
- Bras, J., Guerreiro, R. & Hardy, J. (2012). Use of next-generation sequencing and other whole-genome strategies to dissect neurological disease. *Nat Rev Neurosci*, 13 (7): 453-64.
- Brennan, F., Anderson, A., Taylor, S., Woodruff, T. & Ruitenber, M. (2012). Complement activation in the injured central nervous system: another dual-edged sword? *Journal of Neuroinflammation*, 9 (1): 137.
- Butterfield, D. A., Bader Lange, M. L. & Sultana, R. (2010). Involvements of the lipid peroxidation product, HNE, in the pathogenesis and progression of Alzheimer's disease. *Biochim Biophys Acta*, 1801 (8): 924-9.
- Carlos, T. M., Clark, R. S., Francicola-Higgins, D., Schiding, J. K. & Kochanek, P. M. (1997). Expression of endothelial adhesion molecules and recruitment of neutrophils after traumatic brain injury in rats. *J Leukoc Biol*, 61 (3): 279-85.
- Carlstedt, T. (2008). Root repair review: basic science background and clinical outcome. *Restor Neurol Neurosci*, 26 (2-3): 225-41.
- Castellino, F., Zhong, G. & Germain, R. N. (1997). Antigen Presentation by MHC Class II Molecules: Invariant Chain Function, Protein Trafficking, and the Molecular Basis of Diverse Determinant Capture. *Human Immunology*, 54 (2): 159-169.
- Cernak, I. (2005). Animal models of head trauma. *NeuroRx*, 2 (3): 410-22.
- Chang, C. H., Guerder, S., Hong, S. C., van Ewijk, W. & Flavell, R. A. (1996). Mice lacking the MHC class II transactivator (CIITA) show tissue-specific impairment of MHC class II expression. *Immunity*, 4 (2): 167-78.
- Chastain, E. M., Duncan, D. S., Rodgers, J. M. & Miller, S. D. (2011). The role of antigen presenting cells in multiple sclerosis. *Biochim Biophys Acta*, 1812 (2): 265-74.
- Chen, X. R., Besson, V. C., Palmier, B., Garcia, Y., Plotkine, M. & Marchand-Leroux, C. (2007). Neurological recovery-promoting, anti-inflammatory, and anti-oxidative effects afforded by fenofibrate, a PPAR alpha agonist, in traumatic brain injury. *J Neurotrauma*, 24 (7): 1119-31.
- Chen, Y., Lomnitski, L., Michaelson, D. M. & Shohami, E. (1997). Motor and cognitive deficits in apolipoprotein E-deficient mice after closed head injury. *Neuroscience*, 80 (4): 1255-1262.
- Christman, C. W., Grady, M. S., Walker, S. A., Holloway, K. L. & Povlishock, J. T. (1994). Ultrastructural studies of diffuse axonal injury in humans. *J Neurotrauma*, 11 (2): 173-86.

- Clark, R. S., Carlos, T. M., Schiding, J. K., Bree, M., Fireman, L. A., DeKosky, S. T. & Kochanek, P. M. (1996). Antibodies against Mac-1 attenuate neutrophil accumulation after traumatic brain injury in rats. *J Neurotrauma*, 13 (6): 333-41.
- Clark, R. S. B., Chen, M., Kochanek, P. M., Watkins, S. C., Jin, K. L., Draviam, R., Nathaniel, P. D., Pinto, R., Marion, D. W. & Graham, S. H. (2001). Detection of single- and double-strand DNA breaks after traumatic brain injury in rats: comparison of in situ labeling techniques using DNA polymerase I, the Klenow fragment of DNA polymerase I, and terminal deoxynucleotidyl transferase. *J Neurotrauma*, 18 (7): 675-89.
- Clausen, F. & Hillered, L. (2005). Intracranial pressure changes during fluid percussion, controlled cortical impact and weight drop injury in rats. *Acta Neurochir (Wien)*, 147 (7): 775-80; discussion 780.
- Clausen, F., Lewen, A., Marklund, N., Olsson, Y., McArthur, D. L. & Hillered, L. (2005). Correlation of hippocampal morphological changes and morris water maze performance after cortical contusion injury in rats. *Neurosurgery*, 57 (1): 154-63; discussion 154-63.
- Clausen, F., Lorant, T., Lewen, A. & Hillered, L. (2007). T lymphocyte trafficking: a novel target for neuroprotection in traumatic brain injury. *J Neurotrauma*, 24 (8): 1295-307.
- Clausen, F., Marklund, N., Lewen, A., Enblad, P., Basu, S. & Hillered, L. (2012). Interstitial F(2)-isoprostane 8-iso-PGF(2alpha) as a biomarker of oxidative stress after severe human traumatic brain injury. *J Neurotrauma*, 29 (5): 766-75.
- Cobbs, C. S., Fenoy, A., Bredt, D. S. & Noble, L. J. (1997). Expression of nitric oxide synthase in the cerebral microvasculature after traumatic brain injury in the rat. *Brain Res*, 751 (2): 336-8.
- Comings, D. E. (2003). The real problem in association studies. *Am J Med Genet B Neuropsychiatr Genet*, 116B (1): 102.
- Cook, N. L., Vink, R., Donkin, J. J. & van den Heuvel, C. (2009). Validation of reference genes for normalization of real-time quantitative RT-PCR data in traumatic brain injury. *J Neurosci Res*, 87 (1): 34-41.
- Corbo, R. M. & Scacchi, R. (1999). Apolipoprotein E (APOE) allele distribution in the world. Is APOE*4 a 'thrifty' allele? *Annals of Human Genetics*, 63 (4): 301-310.
- Cornet, A., Bettelli, E., Oukka, M., Cambouris, C., Avellana-Adalid, V., Kosmatopoulos, K. & Liblau, R. S. (2000). Role of astrocytes in antigen presentation and naive T-cell activation. *J Neuroimmunol*, 106 (1-2): 69-77.
- Cox, A. L., Coles, A. J., Nortje, J., Bradley, P. G., Chatfield, D. A., Thompson, S. J. & Menon, D. K. (2006). An investigation of auto-reactivity after head injury. *J Neuroimmunol*, 174 (1-2): 180-6.
- Crawford, F., Wood, M., Ferguson, S., Mathura, V., Gupta, P., Humphrey, J., Mouzon, B., Laporte, V., Margenthaler, E., O'Steen, B., et al. (2009). Apolipoprotein E-genotype dependent hippocampal and cortical responses to traumatic brain injury. *Neuroscience*, 159 (4): 1349-1362.
- Crotzer, V. L. & Blum, J. S. (2010). Autophagy and adaptive immunity. *Immunology*, 131 (1): 9-17.
- Cunningham, C., Wilcockson, D. C., Champion, S., Lunnon, K. & Perry, V. H. (2005). Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J Neurosci*, 25 (40): 9275-84.
- Dalla Libera, A. L., Regner, A., de Paoli, J., Centenaro, L., Martins, T. T. & Simon, D. (2011). IL-6 polymorphism associated with fatal outcome in patients with severe traumatic brain injury. *Brain Inj*, 25 (4): 365-9.
- Dardiotis, E., Fountas, K. N., Dardioti, M., Xiromerisiou, G., Kapsalaki, E., Tasiou, A. & Hadjigeorgiou, G. M. (2010). Genetic association studies in patients with traumatic brain injury. *Neurosurg Focus*, 28 (1): E9.
- Dardiotis, E., Grigoriadis, S. & Hadjigeorgiou, G. M. (2012). Genetic factors influencing outcome from neurotrauma. *Curr Opin Psychiatry*, 25 (3): 231-8.
- Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., Littman, D. R., Dustin, M. L. & Gan, W.-B. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci*, 8 (6): 752-758.
- Davies, A. L., Hayes, K. C. & Dekaban, G. A. (2007). Clinical correlates of elevated serum concentrations of cytokines and autoantibodies in patients with spinal cord injury. *Arch Phys Med Rehabil*, 88 (11): 1384-93.
- de Graaf, K. L., Weissert, R., Kjellen, P., Holmdahl, R. & Olsson, T. (1999). Allelic variations in rat MHC class II binding of myelin basic protein peptides correlate with encephalitogenicity. *Int Immunol*, 11 (12): 1981-8.
- Diaz-Arrastia, R. & Baxter, V. K. (2006). Genetic factors in outcome after traumatic brain injury: what the human genome project can teach us about brain trauma. *J Head Trauma Rehabil*, 21 (4): 361-74.

- Dixon, C. E., Lyeth, B. G., Povlishock, J. T., Findling, R. L., Hamm, R. J., Marmarou, A., Young, H. F. & Hayes, R. L. (1987). A fluid percussion model of experimental brain injury in the rat. *J Neurosurg*, 67 (1): 110-9.
- Dixon, C. E., Clifton, G. L., Lighthall, J. W., Yaghmai, A. A. & Hayes, R. L. (1991). A controlled cortical impact model of traumatic brain injury in the rat. *J Neurosci Methods*, 39 (3): 253-62.
- Dominguez, C. A., Li, L., Lidman, O., Olsson, T., Wiesenfeld-Hallin, Z., Piehl, F. & Xu, X. J. (2008). Both MHC and non-MHC genes regulate development of experimental neuropathic pain in rats. *Neurosci Lett*, 442 (3): 284-6.
- Dorph-Petersen, K. A., Nyengaard, J. R. & Gundersen, H. J. G. (2001). Tissue shrinkage and unbiased stereological estimation of particle number and size*. *Journal of Microscopy*, 204 (3): 232-246.
- Drake, T. A., Schadt, E. E. & Lusis, A. J. (2006). Integrating genetic and gene expression data: application to cardiovascular and metabolic traits in mice. *Mamm Genome*, 17 (6): 466-79.
- Dringen, R. (2000). Metabolism and functions of glutathione in brain. *Prog Neurobiol*, 62 (6): 649-71.
- Edwards, P., Arango, M., Balica, L., Cottingham, R., El-Sayed, H., Farrell, B., Fernandes, J., Gogichaisvili, T., Golden, N., Hartzenberg, B., et al. (2005). Final results of MRC CRASH, a randomised placebo-controlled trial of intravenous corticosteroid in adults with head injury-outcomes at 6 months. *Lancet*, 365 (9475): 1957-9.
- Elias, H., Hennig, A. & Schwartz, D. E. (1971). Stereology: applications to biomedical research. *Physiol Rev*, 51 (1): 158-200.
- Elliott, M. B., Jallo, J. J. & Tuma, R. F. (2008). An investigation of cerebral edema and injury volume assessments for controlled cortical impact injury. *J Neurosci Methods*, 168 (2): 320-4.
- Elward, K. & Gasque, P. (2003). "Eat me" and "don't eat me" signals govern the innate immune response and tissue repair in the CNS: emphasis on the critical role of the complement system. *Mol Immunol*, 40 (2-4): 85-94.
- Engel, S., Schluesener, H., Mittelbronn, M., Seid, K., Adjodah, D., Wehner, H. D. & Meyermann, R. (2000). Dynamics of microglial activation after human traumatic brain injury are revealed by delayed expression of macrophage-related proteins MRP8 and MRP14. *Acta Neuropathol*, 100 (3): 313-22.
- Engle, M. R., Singh, S. P., Czernik, P. J., Gaddy, D., Montague, D. C., Ceci, J. D., Yang, Y., Awasthi, S., Awasthi, Y. C. & Zimniak, P. (2004). Physiological role of mGSTA4-4, a glutathione S-transferase metabolizing 4-hydroxynonenal: generation and analysis of mGsta4 null mouse. *Toxicol Appl Pharmacol*, 194 (3): 296-308.
- Engvall, E. & Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry*, 8 (9): 871-4.
- Fatokun, A. A., Stone, T. W. & Smith, R. A. (2008). Oxidative stress in neurodegeneration and available means of protection. *Front Biosci*, 13: 3288-311.
- Feeney, D. M., Boyeson, M. G., Linn, R. T., Murray, H. M. & Dail, W. G. (1981). Responses to cortical injury: I. Methodology and local effects of contusions in the rat. *Brain Res*, 211 (1): 67-77.
- Finsen, B. & Owens, T. (2011). Innate immune responses in central nervous system inflammation. *FEBS Lett*, 585 (23): 3806-12.
- Fissolo, N., Haag, S., de Graaf, K. L., Drews, O., Stevanovic, S., Rammensee, H. G. & Weissert, R. (2009). Naturally presented peptides on major histocompatibility complex I and II molecules eluted from central nervous system of multiple sclerosis patients. *Mol Cell Proteomics*, 8 (9): 2090-101.
- Flierl, M. A., Stahel, P. F., Beauchamp, K. M., Morgan, S. J., Smith, W. R. & Shohami, E. (2009). Mouse closed head injury model induced by a weight-drop device. *Nat Protoc*, 4 (9): 1328-37.
- Floyd, C. L. & Lyeth, B. G. (2007). Astroglia: Important mediators of traumatic brain injury. In John, T. W. & Andrew, I. R. M. (eds) vol. Volume 161 *Progress in Brain Research*, pp. 61-79: Elsevier.
- Fox, G. B., LeVasseur, R. A. & Faden, A. I. (1999). Behavioral responses of C57BL/6, FVB/N, and 129/SvEMS mouse strains to traumatic brain injury: implications for gene targeting approaches to neurotrauma. *J Neurotrauma*, 16 (5): 377-89.
- Francis, K., van Beek, J., Canova, C., Neal, J. W. & Gasque, P. (2003). Innate immunity and brain inflammation: the key role of complement. *Expert Rev Mol Med*, 5 (15): 1-19.
- Fu, Q., Zhu, J. & Van Eyk, J. E. (2010). Comparison of multiplex immunoassay platforms. *Clin Chem*, 56 (2): 314-8.
- Fukuda, M., Kanou, F., Shimada, N., Sawabe, M., Saito, Y., Murayama, S., Hashimoto, M., Maruyama, N. & Ishigami, A. (2009). Elevated levels of 4-hydroxynonenal-histidine Michael adduct in the hippocampi of patients with Alzheimer's disease. *Biomed Res*, 30 (4): 227-33.
- Gahm, C., Holmin, S. & Mathiesen, T. (2000). Temporal profiles and cellular sources of three nitric oxide synthase isoforms in the brain after experimental contusion. *Neurosurgery*, 46 (1): 169-77.

- Gahm, C., Holmin, S. & Mathiesen, T. (2002). Nitric oxide synthase expression after human brain contusion. *Neurosurgery*, 50 (6): 1319-26.
- Galvani, S., Coatrieux, C., Elbaz, M., Grazide, M. H., Thiers, J. C., Parini, A., Uchida, K., Kamar, N., Rostaing, L., Baltas, M., et al. (2008). Carbonyl scavenger and antiatherogenic effects of hydrazine derivatives. *Free Radic Biol Med*, 45 (10): 1457-67.
- Gandhi, S. & Wood, N. W. (2010). Genome-wide association studies: the key to unlocking neurodegeneration? *Nat Neurosci*, 13 (7): 789-94.
- Gasque, P. (2004). Complement: a unique innate immune sensor for danger signals. *Molecular Immunology*, 41 (11): 1089-1098.
- Gilmer, L. K., Ansari, M. A., Roberts, K. N. & Scheff, S. W. (2010). Age-related mitochondrial changes after traumatic brain injury. *J Neurotrauma*, 27 (5): 939-50.
- Goldacre, M. J., Abisgold, J. D., Yeates, D. G. & Seagroatt, V. (2006). Risk of multiple sclerosis after head injury: record linkage study. *J Neurol Neurosurg Psychiatry*, 77 (3): 351-3.
- Goss, J. R., Taffe, K. M., Kochanek, P. M. & DeKosky, S. T. (1997). The antioxidant enzymes glutathione peroxidase and catalase increase following traumatic brain injury in the rat. *Exp Neurol*, 146 (1): 291-4.
- Gourraud, P.-A., Harbo, H. F., Hauser, S. L. & Baranzini, S. E. (2012). The genetics of multiple sclerosis: an up-to-date review. *Immunological Reviews*, 248 (1): 87-103.
- Griffiths, M. R., Gasque, P. & Neal, J. W. (2009). The multiple roles of the innate immune system in the regulation of apoptosis and inflammation in the brain. *J Neuropathol Exp Neurol*, 68 (3): 217-26.
- Guerini, F. R., Calabrese, E., Agliardi, C., Zanzottera, M., Franceschi, M., Grimaldi, L. M., Nemni, R. & Ferrante, P. (2009). Association study of the HLA-A2 allele in Italian Alzheimer disease patients. *Neurobiol Aging*, 30 (12): 2082-3.
- Gundersen, H. J., Bagger, P., Bendtsen, T. F., Evans, S. M., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., et al. (1988). The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS*, 96 (10): 857-81.
- Gustavsson, A., Svensson, M., Jacobi, F., Allgulander, C., Alonso, J., Beghi, E., Dodel, R., Ekman, M., Faravelli, C., Fratiglioni, L., et al. (2011). Cost of disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol*, 21 (10): 718-79.
- Hadjigeorgiou, G. M., Paterakis, K., Dardiotis, E., Dardioti, M., Aggelakis, K., Tasiou, A., Xiromerisiou, G., Komnos, A., Zintzaras, E., Scarneas, N., et al. (2005). IL-1RN and IL-1B gene polymorphisms and cerebral hemorrhagic events after traumatic brain injury. *Neurology*, 65 (7): 1077-82.
- Hall, E. D., Andrus, P. K. & Yonkers, P. A. (1993). Brain hydroxyl radical generation in acute experimental head injury. *J Neurochem*, 60 (2): 588-94.
- Hall, E. D., Andrus, P. K., Yonkers, P. A., Smith, S. L., Zhang, J. R., Taylor, B. M. & Sun, F. F. (1994). Generation and detection of hydroxyl radical following experimental head injury. *Ann N Y Acad Sci*, 738: 15-24.
- Hall, E. D., Detloff, M. R., Johnson, K. & Kupina, N. C. (2004). Peroxynitrite-mediated protein nitration and lipid peroxidation in a mouse model of traumatic brain injury. *J Neurotrauma*, 21 (1): 9-20.
- Hall, E. D., Vaishnav, R. A. & Mustafa, A. G. (2010). Antioxidant therapies for traumatic brain injury. *Neurotherapeutics*, 7 (1): 51-61.
- Hammarberg, H., Risling, M., Hokfelt, T., Cullheim, S. & Piehl, F. (1998). Expression of insulin-like growth factors and corresponding binding proteins (IGFBP 1-6) in rat spinal cord and peripheral nerve after axonal injuries. *J Comp Neurol*, 400 (1): 57-72.
- Hardy, J. (2002). The real problem in association studies. *Am J Med Genet*, 114 (2): 253.
- Harnesk, K., Swanberg, M., Ockinger, J., Diez, M., Lidman, O., Wallstrom, E., Lobell, A., Olsson, T. & Piehl, F. (2008). Vra4 congenic rats with allelic differences in the class II transactivator gene display altered susceptibility to experimental autoimmune encephalomyelitis. *J Immunol*, 180 (5): 3289-96.
- Hartman, R. E., Laurer, H., Longhi, L., Bales, K. R., Paul, S. M., McIntosh, T. K. & Holtzman, D. M. (2002). Apolipoprotein E4 Influences Amyloid Deposition But Not Cell Loss after Traumatic Brain Injury in a Mouse Model of Alzheimer's Disease. *The Journal of Neuroscience*, 22 (23): 10083-10087.
- Hayes, K. C., Hull, T. C., Delaney, G. A., Potter, P. J., Sequeira, K. A., Campbell, K. & Popovich, P. G. (2002). Elevated serum titers of proinflammatory cytokines and CNS autoantibodies in patients with chronic spinal cord injury. *J Neurotrauma*, 19 (6): 753-61.
- Heid, C. A., Stevens, J., Livak, K. J. & Williams, P. M. (1996). Real time quantitative PCR. *Genome Res*, 6 (10): 986-94.

- Helmy, A., De Simoni, M.-G., Guilfoyle, M. R., Carpenter, K. L. H. & Hutchinson, P. J. (2011). Cytokines and innate inflammation in the pathogenesis of human traumatic brain injury. *Progress in Neurobiology*, 95 (3): 352-372.
- Helmy, A., De Simoni, M. G., Guilfoyle, M. R., Carpenter, K. L. & Hutchinson, P. J. (2011). Cytokines and innate inflammation in the pathogenesis of human traumatic brain injury. *Prog Neurobiol*, 95 (3): 352-72.
- Holmin, S., Mathiesen, T., Shetye, J. & Biberfeld, P. (1995). Intracerebral inflammatory response to experimental brain contusion. *Acta Neurochir (Wien)*, 132 (1-3): 110-9.
- Holmin, S., Soderlund, J., Biberfeld, P. & Mathiesen, T. (1998). Intracerebral inflammation after human brain contusion. *Neurosurgery*, 42 (2): 291-8; discussion 298-9.
- Hurt, P., Walter, L., Sudbrak, R., Klages, S., Muller, I., Shiina, T., Inoko, H., Lehrach, H., Gunther, E., Reinhardt, R., et al. (2004). The genomic sequence and comparative analysis of the rat major histocompatibility complex. *Genome Res*, 14 (4): 631-9.
- Inngjerdigen, M., Kveberg, L. & Vaage, J. T. (2012). A novel NKR-P1B(bright) NK cell subset expresses an activated CD25(+)CX(3)CR1(+)CD62L(-)CD11b(-)CD27(-) phenotype and is prevalent in blood, liver, and gut-associated lymphoid organs of rats. *J Immunol*, 188 (6): 2499-508.
- Irizarry, R. A., Hobbs, B., Collin, F., Beazer-Barclay, Y. D., Antonellis, K. J., Scherf, U. & Speed, T. P. (2003). Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Bioinformatics*, 4 (2): 249-64.
- Ishige, N., Pitts, L. H., Hashimoto, T., Nishimura, M. C. & Bartkowski, H. M. (1987). Effect of hypoxia on traumatic brain injury in rats: Part 1. Changes in neurological function, electroencephalograms, and histopathology. *Neurosurgery*, 20 (6): 848-53.
- Itoh, T., Satou, T., Nishida, S., Tsubaki, M., Imano, M., Hashimoto, S. & Ito, H. (2010). Edaravone protects against apoptotic neuronal cell death and improves cerebral function after traumatic brain injury in rats. *Neurochem Res*, 35 (2): 348-55.
- Johnson, V. E., Murray, L., Raghupathi, R., Stewart, J., Nicoll, J. A., MacKinnon, M. A., McIntosh, T. K. & Graham, D. I. (2006). No evidence for the presence of apolipoprotein epsilon4, interleukin-lalpha allele 2 and interleukin-1beta allele 2 cause an increase in programmed cell death following traumatic brain injury in humans. *Clin Neuropathol*, 25 (6): 255-64.
- Johnson, V. E., Stewart, W., Graham, D. I., Stewart, J. E., Praestgaard, A. H. & Smith, D. H. (2009). A neprilysin polymorphism and amyloid-beta plaques after traumatic brain injury. *J Neurotrauma*, 26 (8): 1197-202.
- Joly, E., Leong, L., Coadwell, W. J., Clarkson, C. & Butcher, G. W. (1996). The rat MHC haplotype RT1c expresses two classical class I molecules. *J Immunol*, 157 (4): 1551-8.
- Jordan, B. D. (2007). Genetic influences on outcome following traumatic brain injury. *Neurochem Res*, 32 (4-5): 905-15.
- Kang, J. H. & Lin, H. C. (2012). Increased risk of multiple sclerosis after traumatic brain injury: a nationwide population-based study. *J Neurotrauma*, 29 (1): 90-5.
- Kasprzak, H. A., Wozniak, A., Drewa, G. & Wozniak, B. (2001). Enhanced lipid peroxidation processes in patients after brain contusion. *J Neurotrauma*, 18 (8): 793-7.
- Keane, R. W., Kraydieh, S., Lotocki, G., Alonso, O. F., Aldana, P. & Dietrich, W. D. (2001). Apoptotic and antiapoptotic mechanisms after traumatic brain injury. *J Cereb Blood Flow Metab*, 21 (10): 1189-98.
- Kenne, E., Erlandsson, A., Lindbom, L., Hillered, L. & Clausen, F. (2012). Neutrophil depletion reduces edema formation and tissue loss following traumatic brain injury in mice. *J Neuroinflammation*, 9: 17.
- Kerman, M., Kanter, M., Coskun, K. K., Erboga, M. & Gurel, A. (2012). Neuroprotective effects of caffeic acid phenethyl ester on experimental traumatic brain injury in rats. *J Mol Histol*, 43 (1): 49-57.
- Kerr, J. F., Wyllie, A. H. & Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*, 26 (4): 239-57.
- Kettenmann, H., Hanisch, U.-K., Noda, M. & Verkhratsky, A. (2011). Physiology of Microglia. *Physiological Reviews*, 91 (2): 461-553.
- Kheradmand, T., Trivedi, P. P., Wolf, N. A., Roberts, P. C. & Swanborg, R. H. (2008). Characterization of a subset of bone marrow-derived natural killer cells that regulates T cell activation in rats. *J Leukoc Biol*, 83 (5): 1128-35.
- Kiernan, J. A. (1985). Axonal and vascular changes following injury to the rat's optic nerve. *J Anat*, 141: 139-54.

- Kim, J., Basak, J. M. & Holtzman, D. M. (2009). The Role of Apolipoprotein E in Alzheimer's Disease. *Neuron*, 63 (3): 287-303.
- Kipnis, J., Mizrahi, T., Hauben, E., Shaked, I., Shevach, E. & Schwartz, M. (2002). Neuroprotective autoimmunity: naturally occurring CD4+CD25+ regulatory T cells suppress the ability to withstand injury to the central nervous system. *Proc Natl Acad Sci U S A*, 99 (24): 15620-5.
- Koliatsos, V. E., Price, W. L., Pardo, C. A. & Price, D. L. (1994). Ventral root avulsion: an experimental model of death of adult motor neurons. *J Comp Neurol*, 342 (1): 35-44.
- Kontos, H. A. & Povlishock, J. T. (1986). Oxygen radicals in brain injury. *Cent Nerv Syst Trauma*, 3 (4): 257-63.
- Kontos, H. A. & Wei, E. P. (1986). Superoxide production in experimental brain injury. *J Neurosurg*, 64 (5): 803-7.
- Kornblum, J. (1968). The application of the irradiated hamster test to the study of experimental allergic encephalomyelitis. *J Immunol*, 101 (4): 702-10.
- Kors, E. E., Terwindt, G. M., Vermeulen, F. L., Fitzsimons, R. B., Jardine, P. E., Heywood, P., Love, S., van den Maagdenberg, A. M., Haan, J., Frants, R. R., et al. (2001). Delayed cerebral edema and fatal coma after minor head trauma: role of the CACNA1A calcium channel subunit gene and relationship with familial hemiplegic migraine. *Ann Neurol*, 49 (6): 753-60.
- Kossmann, T., Stahel, P. F., Morganti-Kossmann, M. C., Jones, J. L. & Barnum, S. R. (1997). Elevated levels of the complement components C3 and factor B in ventricular cerebrospinal fluid of patients with traumatic brain injury. *J Neuroimmunol*, 73 (1-2): 63-9.
- Krueger, F., Pardini, M., Huey, E. D., Raymont, V., Solomon, J., Lipsky, R. H., Hodgkinson, C. A., Goldman, D. & Grafman, J. (2011). The role of the Met66 brain-derived neurotrophic factor allele in the recovery of executive functioning after combat-related traumatic brain injury. *J Neurosci*, 31 (2): 598-606.
- Kurien, B. T. & Scofield, R. H. (2008). Autoimmunity and oxidatively modified autoantigens. *Autoimmun Rev*, 7 (7): 567-73.
- Kurowski, B., Martin, L. J. & Wade, S. L. (2012). Genetics and outcomes after traumatic brain injury (TBI): What do we know about pediatric TBI? *J Pediatr Rehabil Med*, 5 (3): 217-31.
- Kurts, C., Robinson, B. W. S. & Knolle, P. A. (2010). Cross-priming in health and disease. *Nat Rev Immunol*, 10 (6): 403-414.
- Landi, S. (2000). Mammalian class theta GST and differential susceptibility to carcinogens: a review. *Mutat Res*, 463 (3): 247-83.
- Lee, S., Lee, J., Kim, S., Park, J. Y., Lee, W. H., Mori, K., Kim, S. H., Kim, I. K. & Suk, K. (2007). A dual role of lipocalin 2 in the apoptosis and deramification of activated microglia. *J Immunol*, 179 (5): 3231-41.
- Lee, S., Park, J. Y., Lee, W. H., Kim, H., Park, H. C., Mori, K. & Suk, K. (2009). Lipocalin-2 is an autocrine mediator of reactive astrocytosis. *J Neurosci*, 29 (1): 234-49.
- Leinhase, I., Holers, V. M., Thurman, J. M., Harhausen, D., Schmidt, O. I., Pietzcker, M., Taha, M. E., Rittirsch, D., Huber-Lang, M., Smith, W. R., et al. (2006a). Reduced neuronal cell death after experimental brain injury in mice lacking a functional alternative pathway of complement activation. *BMC Neurosci*, 7: 55.
- Leinhase, I., Schmidt, O. I., Thurman, J. M., Hossini, A. M., Rozanski, M., Taha, M. E., Scheffler, A., John, T., Smith, W. R., Holers, V. M., et al. (2006b). Pharmacological complement inhibition at the C3 convertase level promotes neuronal survival, neuroprotective intracerebral gene expression, and neurological outcome after traumatic brain injury. *Experimental Neurology*, 199 (2): 454-464.
- Leinhase, I., Rozanski, M., Harhausen, D., Thurman, J. M., Schmidt, O. I., Hossini, A. M., Taha, M. E., Rittirsch, D., Ward, P. A., Holers, V. M., et al. (2007). Inhibition of the alternative complement activation pathway in traumatic brain injury by a monoclonal anti-factor B antibody: a randomized placebo-controlled study in mice. *J Neuroinflammation*, 4: 13.
- Lewen, A., Li, G. L., Olsson, Y. & Hillered, L. (1996). Changes in microtubule-associated protein 2 and amyloid precursor protein immunoreactivity following traumatic brain injury in rat: influence of MK-801 treatment. *Brain Res*, 719 (1-2): 161-71.
- Lidman, O., Swanberg, M., Horvath, L., Broman, K. W., Olsson, T. & Piehl, F. (2003). Discrete gene loci regulate neurodegeneration, lymphocyte infiltration, and major histocompatibility complex class II expression in the CNS. *J Neurosci*, 23 (30): 9817-23.
- Lingsma, H. F., Roozenbeek, B., Steyerberg, E. W., Murray, G. D. & Maas, A. I. (2010). Early prognosis in traumatic brain injury: from prophecies to predictions. *Lancet Neurol*, 9 (5): 543-54.

- Linker, R. A., Lee, D. H., Ryan, S., van Dam, A. M., Conrad, R., Bista, P., Zeng, W., Hronowsky, X., Buko, A., Chollate, S., et al. (2011). Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain*, 134 (Pt 3): 678-92.
- Lipsky, R. H., Sparling, M. B., Ryan, L. M., Xu, K., Salazar, A. M., Goldman, D. & Warden, D. L. (2005). Association of COMT Val158Met genotype with executive functioning following traumatic brain injury. *J Neuropsychiatry Clin Neurosci*, 17 (4): 465-71.
- Loane, D. J. & Byrnes, K. R. (2010). Role of microglia in neurotrauma. *Neurotherapeutics*, 7 (4): 366-77.
- Lockhart, D. J., Dong, H., Byrne, M. C., Follettie, M. T., Gallo, M. V., Chee, M. S., Mittmann, M., Wang, C., Kobayashi, M., Horton, H., et al. (1996). Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol*, 14 (13): 1675-80.
- Lomnitski, L., Kohen, R., Chen, Y., Shohami, E., Trembovler, V., Vogel, T. & Michaelson, D. M. (1997). Reduced Levels of Antioxidants in Brains of Apolipoprotein E-Deficient Mice Following Closed Head Injury. *Pharmacology Biochemistry and Behavior*, 56 (4): 669-673.
- Longhi, L., Perego, C., Ortolano, F., Zanier, E. R., Bianchi, P., Stocchetti, N., McIntosh, T. K. & De Simoni, M. G. (2009). C1-inhibitor attenuates neurobehavioral deficits and reduces contusion volume after controlled cortical impact brain injury in mice. *Crit Care Med*, 37 (2): 659-65.
- Lopez-Escribano, H., Minambres, E., Labrador, M., Bartolome, M. J. & Lopez-Hoyos, M. (2002). Induction of cell death by sera from patients with acute brain injury as a mechanism of production of autoantibodies. *Arthritis Rheum*, 46 (12): 3290-300.
- Lucin, K. M., Sanders, V. M., Jones, T. B., Malarkey, W. B. & Popovich, P. G. (2007). Impaired antibody synthesis after spinal cord injury is level dependent and is due to sympathetic nervous system dysregulation. *Exp Neurol*, 207 (1): 75-84.
- Lundberg, C., Lidman, O., Holmdahl, R., Olsson, T. & Piehl, F. (2001). Neurodegeneration and glial activation patterns after mechanical nerve injury are differentially regulated by non-MHC genes in congenic inbred rat strains. *J Comp Neurol*, 431 (1): 75-87.
- Luo, C. L., Li, B. X., Li, Q. Q., Chen, X. P., Sun, Y. X., Bao, H. J., Dai, D. K., Shen, Y. W., Xu, H. F., Ni, H., et al. (2011). Autophagy is involved in traumatic brain injury-induced cell death and contributes to functional outcome deficits in mice. *Neuroscience*, 184: 54-63.
- Lynch, J. R., Pineda, J. A., Morgan, D., Zhang, L., Warner, D. S., Benveniste, H. & Laskowitz, D. T. (2002). Apolipoprotein E affects the central nervous system response to injury and the development of cerebral edema. *Annals of Neurology*, 51 (1): 113-117.
- Maas, A. I., Roozenbeek, B. & Manley, G. T. (2010a). Clinical trials in traumatic brain injury: past experience and current developments. *Neurotherapeutics*, 7 (1): 115-26.
- Maas, A. I., Steyerberg, E. W., Marmarou, A., McHugh, G. S., Lingsma, H. F., Butcher, I., Lu, J., Weir, J., Roozenbeek, B. & Murray, G. D. (2010b). IMPACT recommendations for improving the design and analysis of clinical trials in moderate to severe traumatic brain injury. *Neurotherapeutics*, 7 (1): 127-34.
- Mahley, R. W. & Rall, S. C. (2000). APOLIPOPROTEIN E: Far More Than a Lipid Transport Protein. *Annual Review of Genomics and Human Genetics*, 1 (1): 507-537.
- Marklund, N., Clausen, F., Lewen, A., Hovda, D. A., Olsson, Y. & Hillered, L. (2001). alpha-Phenyl-tert-N-butyl nitron (PBN) improves functional and morphological outcome after cortical contusion injury in the rat. *Acta Neurochir (Wien)*, 143 (1): 73-81.
- Marklund, N., Bakshi, A., Castelbuono, D. J., Conte, V. & McIntosh, T. K. (2006). Evaluation of pharmacological treatment strategies in traumatic brain injury. *Curr Pharm Des*, 12 (13): 1645-80.
- Marklund, N. & Hillered, L. (2011). Animal modelling of traumatic brain injury in preclinical drug development: where do we go from here? *Br J Pharmacol*, 164 (4): 1207-29.
- Marmarou, A., Foda, M. A., van den Brink, W., Campbell, J., Kita, H. & Demetriadou, K. (1994). A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *J Neurosurg*, 80 (2): 291-300.
- Martinez-Lucas, P., Moreno-Cuesta, J., Garcia-Olmo, D. C., Sanchez-Sanchez, F., Escibano-Martinez, J., del Pozo, A. C., Lizan-Garcia, M. & Garcia-Olmo, D. (2005). Relationship between the Arg72Pro polymorphism of p53 and outcome for patients with traumatic brain injury. *Intensive Care Med*, 31 (9): 1168-73.
- Martinez, F. O., Sica, A., Mantovani, A. & Locati, M. (2008). Macrophage activation and polarization. *Front Biosci*, 13: 453-61.
- McAllister, T. W., Rhodes, C. H., Flashman, L. A., McDonald, B. C., Belloni, D. & Saykin, A. J. (2005). Effect of the dopamine D2 receptor T allele on response latency after mild traumatic brain injury. *Am J Psychiatry*, 162 (9): 1749-51.

- McAllister, T. W., Tyler, A. L., Flashman, L. A., Rhodes, C. H., McDonald, B. C., Saykin, A. J., Tosteson, T. D., Tsongalis, G. J. & Moore, J. H. (2012). Polymorphisms in the brain-derived neurotrophic factor gene influence memory and processing speed one month after brain injury. *J Neurotrauma*, 29 (6): 1111-8.
- McCracken, E., Valeriani, V., Simpson, C., Jover, T., McCulloch, J. & Dewar, D. (2000). The lipid peroxidation by-product 4-hydroxynonenal is toxic to axons and oligodendrocytes. *J Cereb Blood Flow Metab*, 20 (11): 1529-36.
- McGraw, J., Hiebert, G. W. & Steeves, J. D. (2001). Modulating astrogliosis after neurotrauma. *Journal of Neuroscience Research*, 63 (2): 109-115.
- McIntosh, T. K., Noble, L., Andrews, B. & Faden, A. I. (1987). Traumatic brain injury in the rat: characterization of a midline fluid-percussion model. *Cent Nerv Syst Trauma*, 4 (2): 119-34.
- McIntosh, T. K., Vink, R., Noble, L., Yamakami, I., Fernyak, S., Soares, H. & Faden, A. L. (1989). Traumatic brain injury in the rat: characterization of a lateral fluid-percussion model. *Neuroscience*, 28 (1): 233-44.
- McKimmie, C. S. & Graham, G. J. (2010). Astrocytes modulate the chemokine network in a pathogen-specific manner. *Biochemical and Biophysical Research Communications*, 394 (4): 1006-1011.
- Mikawa, S., Kinouchi, H., Kamii, H., Gobbel, G. T., Chen, S. F., Carlson, E., Epstein, C. J. & Chan, P. H. (1996). Attenuation of acute and chronic damage following traumatic brain injury in copper, zinc-superoxide dismutase transgenic mice. *J Neurosurg*, 85 (5): 885-91.
- Morgan, B. P., Gasque, P., Singhrao, S. & Piddlesden, S. J. (1997). The role of complement in disorders of the nervous system. *Immunopharmacology*, 38 (1-2): 43-50.
- Morganti-Kossmann, M. C., Rancan, M., Stahel, P. F. & Kossmann, T. (2002). Inflammatory response in acute traumatic brain injury: a double-edged sword. *Curr Opin Crit Care*, 8 (2): 101-5.
- Morris, M., Uchida, K. & Do, T. (2006). A magnetic torsional wave near the Galactic Centre traced by a 'double helix' nebula. *Nature*, 440 (7082): 308-10.
- Morrow, J. D., Hill, K. E., Burk, R. F., Nammour, T. M., Badr, K. F. & Roberts, L. J., 2nd. (1990). A series of prostaglandin F₂-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci U S A*, 87 (23): 9383-7.
- Muhlethaler-Mottet, A., Otten, L. A., Steimle, V. & Mach, B. (1997). Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. *EMBO J*, 16 (10): 2851-60.
- Nagamoto-Combs, K., Morecraft, R. J., Darling, W. G. & Combs, C. K. (2010). Long-term gliosis and molecular changes in the cervical spinal cord of the rhesus monkey after traumatic brain injury. *J Neurotrauma*, 27 (3): 565-85.
- Naj, A. C., Jun, G., Beecham, G. W., Wang, L. S., Vardarajan, B. N., Buros, J., Gallins, P. J., Buxbaum, J. D., Jarvik, G. P., Crane, P. K., et al. (2011). Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet*, 43 (5): 436-41.
- Neefjes, J., Jongasma, M. L. M., Paul, P. & Bakke, O. (2011). Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol*, 11 (12): 823-836.
- Newcomb, J. K., Zhao, X., Pike, B. R. & Hayes, R. L. (1999). Temporal profile of apoptotic-like changes in neurons and astrocytes following controlled cortical impact injury in the rat. *Exp Neurol*, 158 (1): 76-88.
- Ng, I., Yeo, T. T., Tang, W. Y., Soong, R., Ng, P. Y. & Smith, D. R. (2000). Apoptosis occurs after cerebral contusions in humans. *Neurosurgery*, 46 (4): 949-56.
- Nielsen, H. H., Ladeby, R., Fenger, C., Toft-Hansen, H., Babcock, A. A., Owens, T. & Finsen, B. (2009). Enhanced microglial clearance of myelin debris in T cell-infiltrated central nervous system. *J Neuropathol Exp Neurol*, 68 (8): 845-56.
- Norgren, N., Sundstrom, P., Svenningsson, A., Rosengren, L., Stigbrand, T. & Gunnarsson, M. (2004). Neurofilament and glial fibrillary acidic protein in multiple sclerosis. *Neurology*, 63 (9): 1586-90.
- Norgren, N., Edelstam, A. & Stigbrand, T. (2005). Cerebrospinal fluid levels of neurofilament light in chronic experimental autoimmune encephalomyelitis. *Brain Res Bull*, 67 (4): 264-8.
- O'Connor, W. T., Smyth, A. & Gilchrist, M. D. (2011). Animal models of traumatic brain injury: a critical evaluation. *Pharmacol Ther*, 130 (2): 106-13.
- Oehmichen, M., Jakob, S., Mann, S., Saternus, K. S., Pedal, I. & Meissner, C. (2009). Macrophage subsets in mechanical brain injury (MBI)--a contribution to timing of MBI based on immunohistochemical methods: a pilot study. *Leg Med (Tokyo)*, 11 (3): 118-24.
- Olsson, T., Lundberg, C., Lidman, O. & Piehl, F. (2000). Genetic regulation of nerve avulsion-induced spinal cord inflammation. *Ann N Y Acad Sci*, 917: 186-96.

- Pedersen, W. A., Fu, W., Keller, J. N., Markesbery, W. R., Appel, S., Smith, R. G., Kasarskis, E. & Mattson, M. P. (1998). Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients. *Ann Neurol*, 44 (5): 819-24.
- Penas, C., Casas, C., Robert, I., Fores, J. & Navarro, X. (2009). Cytoskeletal and activity-related changes in spinal motoneurons after root avulsion. *J Neurotrauma*, 26 (5): 763-79.
- Perluigi, M., Fai Poon, H., Hensley, K., Pierce, W. M., Klein, J. B., Calabrese, V., De Marco, C. & Butterfield, D. A. (2005). Proteomic analysis of 4-hydroxy-2-nonenal-modified proteins in G93A-SOD1 transgenic mice--a model of familial amyotrophic lateral sclerosis. *Free Radic Biol Med*, 38 (7): 960-8.
- Perry, V. H. & O'Connor, V. (2008). C1q: the perfect complement for a synaptic feast? *Nat Rev Neurosci*, 9 (11): 807-11.
- Pfleger, C. C., Koch-Henriksen, N., Stenager, E., Flachs, E. M. & Johansen, C. (2009). Head injury is not a risk factor for multiple sclerosis: a prospective cohort study. *Mult Scler*, 15 (3): 294-8.
- Piehl, F. & Lidman, O. (2001). Neuroinflammation in the rat--CNS cells and their role in the regulation of immune reactions. *Immunol Rev*, 184: 212-25.
- Plomin, R., Haworth, C. M. & Davis, O. S. (2009). Common disorders are quantitative traits. *Nat Rev Genet*, 10 (12): 872-8.
- Popovich, P. G., Stokes, B. T. & Whitacre, C. C. (1996). Concept of autoimmunity following spinal cord injury: possible roles for T lymphocytes in the traumatized central nervous system. *J Neurosci Res*, 45 (4): 349-63.
- Porritt, M. J., Andersson, H. C., Hou, L., Nilsson, A., Pekna, M., Pekny, M. & Nilsson, M. (2012). Photothrombosis-induced infarction of the mouse cerebral cortex is not affected by the Nrf2-activator sulforaphane. *PLoS One*, 7 (7): e41090.
- Poser, C. M. (1994). The role of trauma in the pathogenesis of multiple sclerosis: a review. *Clin Neurol Neurosurg*, 96 (2): 103-10.
- Posmantur, R., Hayes, R. L., Dixon, C. E. & Taft, W. C. (1994). Neurofilament 68 and neurofilament 200 protein levels decrease after traumatic brain injury. *J Neurotrauma*, 11 (5): 533-45.
- Posmantur, R. M., Newcomb, J. K., Kampfl, A. & Hayes, R. L. (2000). Light and confocal microscopic studies of evolutionary changes in neurofilament proteins following cortical impact injury in the rat. *Exp Neurol*, 161 (1): 15-26.
- Pritchard, J. K. & Cox, N. J. (2002). The allelic architecture of human disease genes: common disease-common variant...or not? *Hum Mol Genet*, 11 (20): 2417-23.
- Prochazka, M., Voltnerova, M. & Stefan, J. (1971). Studies of immunologic reactions after brain injury. II. Antibodies against brain tissue lipids after blunt head injury in man. *Int Surg*, 55 (5): 322-6.
- Raghupathi, R. (2004). Cell death mechanisms following traumatic brain injury. *Brain Pathol*, 14 (2): 215-22.
- Ramos, R. G. & Olden, K. (2008). Gene-environment interactions in the development of complex disease phenotypes. *Int J Environ Res Public Health*, 5 (1): 4-11.
- Ransohoff, R. M. & Perry, V. H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu Rev Immunol*, 27: 119-45.
- Rathore, K. I., Berard, J. L., Redensek, A., Chierzi, S., Lopez-Vales, R., Santos, M., Akira, S. & David, S. (2011). Lipocalin 2 plays an immunomodulatory role and has detrimental effects after spinal cord injury. *J Neurosci*, 31 (38): 13412-9.
- Readnower, R. D., Chavko, M., Adeeb, S., Conroy, M. D., Pauly, J. R., McCarron, R. M. & Sullivan, P. G. (2010). Increase in blood-brain barrier permeability, oxidative stress, and activated microglia in a rat model of blast-induced traumatic brain injury. *J Neurosci Res*, 88 (16): 3530-9.
- Reid, W. M., Rolfe, A., Register, D., Lévassieur, J. E., Churn, S. B. & Sun, D. (2010). Strain-related differences after experimental traumatic brain injury in rats. *J Neurotrauma*, 27 (7): 1243-53.
- Ricklin, D., Hajishengallis, G., Yang, K. & Lambris, J. D. (2010). Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*, 11 (9): 785-97.
- Ridet, J. L., Malhotra, S. K., Privat, A. & Gage, F. H. (1997). Reactive astrocytes: Cellular and molecular cues to biological function. *Trends in Neurosciences*, 20 (12): 570-577.
- Rink, A., Fung, K. M., Trojanowski, J. Q., Lee, V. M., Neugebauer, E. & McIntosh, T. K. (1995). Evidence of apoptotic cell death after experimental traumatic brain injury in the rat. *Am J Pathol*, 147 (6): 1575-83.
- Risling, M., Plantman, S., Angeria, M., Rostami, E., Bellander, B. M., Kirkegaard, M., Arborelius, U. & Davidsson, J. (2011). Mechanisms of blast induced brain injuries, experimental studies in rats. *Neuroimage*, 54 Suppl 1: S89-97.
- Roberts, I., Yates, D., Sandercock, P., Farrell, B., Wasserberg, J., Lomas, G., Cottingham, R., Svoboda, P., Brayley, N., Mazairac, G., et al. (2004). Effect of intravenous corticosteroids on death within

- 14 days in 10008 adults with clinically significant head injury (MRC CRASH trial): randomised placebo-controlled trial. *Lancet*, 364 (9442): 1321-8.
- Robertson, C. S., Gopinath, S. P., Valadka, A. B., Van, M., Swank, P. R. & Goodman, J. C. (2011). Variants of the endothelial nitric oxide gene and cerebral blood flow after severe traumatic brain injury. *J Neurotrauma*, 28 (5): 727-37.
- Rostami, E., Krueger, F., Zoubak, S., Dal Monte, O., Raymont, V., Pardini, M., Hodgkinson, C. A., Goldman, D., Risling, M. & Grafman, J. (2011). BDNF polymorphism predicts general intelligence after penetrating traumatic brain injury. *PLoS One*, 6 (11): e27389.
- Rudehill, S., Muhallab, S., Wennersten, A., von Gertten, C., Al Nimer, F., Sandberg-Nordqvist, A. C., Holmin, S. & Mathiesen, T. (2006). Autoreactive antibodies against neurons and basal lamina found in serum following experimental brain contusion in rats. *Acta Neurochir (Wien)*, 148 (2): 199-205; discussion 205.
- Saijo, K. & Glass, C. K. (2011). Microglial cell origin and phenotypes in health and disease. *Nat Rev Immunol*, 11 (11): 775-87.
- Salzer, J., Svenningsson, A. & Sundstrom, P. (2010). Neurofilament light as a prognostic marker in multiple sclerosis. *Mult Scler*, 16 (3): 287-92.
- Santos, A., Borges, N., Cerejo, A., Sarmiento, A. & Azevedo, I. (2005). Catalase activity and thiobarbituric acid reactive substances (TBARS) production in a rat model of diffuse axonal injury. Effect of gadolinium and amiloride. *Neurochem Res*, 30 (5): 625-31.
- Saraiva, A. L., Ferreira, A. P., Silva, L. F., Hoffmann, M. S., Dutra, F. D., Furian, A. F., Oliveira, M. S., Figuera, M. R. & Royes, L. F. (2012). Creatine reduces oxidative stress markers but does not protect against seizure susceptibility after severe traumatic brain injury. *Brain Res Bull*, 87 (2-3): 180-6.
- Sarnaik, A. A., Conley, Y. P., Okonkwo, D. O., Barr, T. L., Fink, E. L., Szabo, C., Kochanek, P. M. & Clark, R. S. (2010). Influence of PARP-1 polymorphisms in patients after traumatic brain injury. *J Neurotrauma*, 27 (3): 465-71.
- Sawcer, S. (2010). Bayes factors in complex genetics. *Eur J Hum Genet*, 18 (7): 746-50.
- Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C. C., Patsopoulos, N. A., Moutsianas, L., Dilthey, A., Su, Z., Freeman, C., Hunt, S. E., et al. (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*, 476 (7359): 214-9.
- Schmitz, C. & Hof, P. R. (2005). Design-based stereology in neuroscience. *Neuroscience*, 130 (4): 813-831.
- Schwab, J. M., Seid, K. & Schluesener, H. J. (2001). Traumatic brain injury induces prolonged accumulation of cyclooxygenase-1 expressing microglia/brain macrophages in rats. *J Neurotrauma*, 18 (9): 881-90.
- Schwartz, M. & Kipnis, J. (2001). Protective autoimmunity: regulation and prospects for vaccination after brain and spinal cord injuries. *Trends Mol Med*, 7 (6): 252-8.
- Scofield, R. H., Kurien, B. T., Ganick, S., McClain, M. T., Pye, Q., James, J. A., Schneider, R. I., Broyles, R. H., Bachmann, M. & Hensley, K. (2005). Modification of lupus-associated 60-kDa Ro protein with the lipid oxidation product 4-hydroxy-2-nonenal increases antigenicity and facilitates epitope spreading. *Free Radic Biol Med*, 38 (6): 719-28.
- Selley, M. L. (1998). (E)-4-hydroxy-2-nonenal may be involved in the pathogenesis of Parkinson's disease. *Free Radic Biol Med*, 25 (2): 169-74.
- Selley, M. L., Close, D. R. & Stern, S. E. (2002). The effect of increased concentrations of homocysteine on the concentration of (E)-4-hydroxy-2-nonenal in the plasma and cerebrospinal fluid of patients with Alzheimer's disease. *Neurobiol Aging*, 23 (3): 383-8.
- Sewell, D. L., Nacewicz, B., Liu, F., Macvilay, S., Erdei, A., Lambris, J. D., Sandor, M. & Fabry, Z. (2004). Complement C3 and C5 play critical roles in traumatic brain injury: blocking effects on neutrophil extravasation by C5a receptor antagonist. *J Neuroimmunol*, 155 (1-2): 55-63.
- Shao, C., Roberts, K. N., Markesbery, W. R., Scheff, S. W. & Lovell, M. A. (2006). Oxidative stress in head trauma in aging. *Free Radic Biol Med*, 41 (1): 77-85.
- Sharma, S., Ying, Z. & Gomez-Pinilla, F. (2010). A pyrazole curcumin derivative restores membrane homeostasis disrupted after brain trauma. *Exp Neurol*, 226 (1): 191-9.
- Shechter, R., Raposo, C., London, A., Sagi, I. & Schwartz, M. (2011). The glial scar-monocyte interplay: a pivotal resolution phase in spinal cord repair. *PLoS One*, 6 (12): e27969.
- Sheehan, D., Meade, G., Foley, V. M. & Dowd, C. A. (2001). Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. *Biochem J*, 360 (Pt 1): 1-16.
- Siems, W. & Grune, T. (2003). Intracellular metabolism of 4-hydroxynonenal. *Mol Aspects Med*, 24 (4-5): 167-75.

- Siman, R., Toraskar, N., Dang, A., McNeil, E., McGarvey, M., Plaum, J., Maloney, E. & Grady, M. S. (2009). A panel of neuron-enriched proteins as markers for traumatic brain injury in humans. *J Neurotrauma*, 26 (11): 1867-77.
- Simpson, E. P., Henry, Y. K., Henkel, J. S., Smith, R. G. & Appel, S. H. (2004). Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden. *Neurology*, 62 (10): 1758-65.
- Sivanandam, T. M. & Thakur, M. K. (2012). Traumatic brain injury: A risk factor for Alzheimer's disease. *Neuroscience & Biobehavioral Reviews*, 36 (5): 1376-1381.
- Skoda, D., Kranda, K., Bojar, M., Glosova, L., Baurle, J., Kenney, J., Romportl, D., Pelichovska, M. & Cvachovec, K. (2006). Antibody formation against beta-tubulin class III in response to brain trauma. *Brain Res Bull*, 68 (4): 213-6.
- Smith, S. L., Andrus, P. K., Zhang, J. R. & Hall, E. D. (1994). Direct measurement of hydroxyl radicals, lipid peroxidation, and blood-brain barrier disruption following unilateral cortical impact head injury in the rat. *J Neurotrauma*, 11 (4): 393-404.
- Soares, H. D., Hicks, R. R., Smith, D. & McIntosh, T. K. (1995). Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury. *J Neurosci*, 15 (12): 8223-33.
- Sosa, R. A. & Forsthuber, T. G. (2011). The critical role of antigen-presentation-induced cytokine crosstalk in the central nervous system in multiple sclerosis and experimental autoimmune encephalomyelitis. *J Interferon Cytokine Res*, 31 (10): 753-68.
- Stahel, P. F., Morganti-Kossmann, M. C. & Kossmann, T. (1998). The role of the complement system in traumatic brain injury. *Brain Res Brain Res Rev*, 27 (3): 243-56.
- Stahel, P. F., Kariya, K., Shohami, E., Barnum, S. R., Eugster, H. P., Trentz, O., Kossmann, T. & Morganti-Kossmann, M. C. (2000). Intracerebral complement C5a receptor (CD88) expression is regulated by TNF and lymphotoxin- α following closed head injury in mice. *Journal of Neuroimmunology*, 109 (2): 164-172.
- Stahel, P. F., Morganti-Kossmann, M. C., Perez, D., Redaelli, C., Gloor, B., Trentz, O. & Kossmann, T. (2001). Intrathecal levels of complement-derived soluble membrane attack complex (sC5b-9) correlate with blood-brain barrier dysfunction in patients with traumatic brain injury. *J Neurotrauma*, 18 (8): 773-81.
- Steimle, V., Otten, L. A., Zufferey, M. & Mach, B. (1993). Complement cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). *Cell*, 75 (1): 135-46.
- Stirling, D. P. & Yong, V. W. (2008). Dynamics of the inflammatory response after murine spinal cord injury revealed by flow cytometry. *J Neurosci Res*, 86 (9): 1944-58.
- Stoica, B. A. & Faden, A. I. (2010). Cell death mechanisms and modulation in traumatic brain injury. *Neurotherapeutics*, 7 (1): 3-12.
- Stuve, O., Youssef, S., Slavin, A. J., King, C. L., Patarroyo, J. C., Hirschberg, D. L., Brickey, W. J., Soos, J. M., Piskurich, J. F., Chapman, H. A., et al. (2002). The role of the MHC class II transactivator in class II expression and antigen presentation by astrocytes and in susceptibility to central nervous system autoimmune disease. *J Immunol*, 169 (12): 6720-32.
- Sultana, R., Perluigi, M. & Allan Butterfield, D. (2012). Lipid peroxidation triggers neurodegeneration: A redox proteomics view into the Alzheimer disease brain. *Free Radic Biol Med*.
- Swanberg, M., Lidman, O., Padyukov, L., Eriksson, P., Akesson, E., Jagodic, M., Lobell, A., Khademi, M., Borjesson, O., Lindgren, C. M., et al. (2005). MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. *Nat Genet*, 37 (5): 486-94.
- Swanberg, M., Harnesk, K., Strom, M., Diez, M., Lidman, O. & Piehl, F. (2009). Fine mapping of gene regions regulating neurodegeneration. *PLoS One*, 4 (6): e5906.
- Szmydynger-Chodobska, J., Strazielle, N., Zink, B. J., Ghersi-Egea, J. F. & Chodobski, A. (2009). The role of the choroid plexus in neutrophil invasion after traumatic brain injury. *J Cereb Blood Flow Metab*, 29 (9): 1503-16.
- Tan, A. A., Quigley, A., Smith, D. C. & Hoane, M. R. (2009). Strain differences in response to traumatic brain injury in Long-Evans compared to Sprague-Dawley rats. *J Neurotrauma*, 26 (4): 539-48.
- Tang, S. C., Arumugam, T. V., Cutler, R. G., Jo, D. G., Magnus, T., Chan, S. L., Mughal, M. R., Telljohann, R. S., Nassar, M., Ouyang, X., et al. (2007). Neuroprotective actions of a histidine analogue in models of ischemic stroke. *J Neurochem*, 101 (3): 729-36.
- Tanriverdi, T., Uzan, M., Sanus, G. Z., Baykara, O., Is, M., Ozkara, C. & Buyra, N. (2006). Lack of association between the IL1A gene (-889) polymorphism and outcome after head injury. *Surgical Neurology*, 65 (1): 7-10.

- Teunissen, C. E., Dijkstra, C. & Polman, C. (2005). Biological markers in CSF and blood for axonal degeneration in multiple sclerosis. *Lancet Neurol*, 4 (1): 32-41.
- Ting, J. P. & Trowsdale, J. (2002). Genetic control of MHC class II expression. *Cell*, 109 Suppl: S21-33.
- Tjoa, T., Strausbaugh, H. J., Maida, N., Dazin, P. F., Rosen, S. D. & Noble-Haeusslein, L. J. (2003). The use of flow cytometry to assess neutrophil infiltration in the injured murine spinal cord. *Journal of Neuroscience Methods*, 129 (1): 49-59.
- Toklu, H. Z., Hakan, T., Biber, N., Solakoglu, S., Ogunc, A. V. & Sener, G. (2009). The protective effect of alpha lipoic acid against traumatic brain injury in rats. *Free Radic Res*, 43 (7): 658-67.
- Tortelli, R., Ruggieri, M., Cortese, R., D'Errico, E., Capozzo, R., Leo, A., Mastrapasqua, M., Zoccolella, S., Leante, R., Livrea, P., et al. (2012). Elevated cerebrospinal fluid neurofilament light levels in patients with amyotrophic lateral sclerosis: a possible marker of disease severity and progression. *Eur J Neurol*.
- Townsend, D. M. & Tew, K. D. (2003). The role of glutathione-S-transferase in anti-cancer drug resistance. *Oncogene*, 22 (47): 7369-75.
- Tuncel, J., Haag, S., Carlsen, S., Yau, A. C., Lu, S., Burkhardt, H. & Holmdahl, R. (2012). Class II major histocompatibility complex-associated response to type XI collagen regulates the development of chronic arthritis in rats. *Arthritis Rheum*, 64 (8): 2537-47.
- Tyurin, V. A., Tyurina, Y. Y., Borisenko, G. G., Sokolova, T. V., Ritov, V. B., Quinn, P. J., Rose, M., Kochanek, P., Graham, S. H. & Kagan, V. E. (2000). Oxidative stress following traumatic brain injury in rats: quantitation of biomarkers and detection of free radical intermediates. *J Neurochem*, 75 (5): 2178-89.
- Uzan, M., Tanriverdi, T., Baykara, O., Kafadar, A., Sanus, G. Z., Tureci, E., Ozkara, C., Uysal, O. & Buyra, N. (2005). Association between interleukin-1 beta (IL-1beta) gene polymorphism and outcome after head injury: an early report. *Acta Neurochir (Wien)*, 147 (7): 715-20; discussion 720.
- Wakeland, E., Morel, L., Achey, K., Yui, M. & Longmate, J. (1997). Speed congenics: a classic technique in the fast lane (relatively speaking). *Immunol Today*, 18 (10): 472-7.
- Wallberg, M., Bergquist, J., Achour, A., Breij, E. & Harris, R. A. (2007). Malondialdehyde modification of myelin oligodendrocyte glycoprotein leads to increased immunogenicity and encephalitogenicity. *Eur J Immunol*, 37 (7): 1986-95.
- van Beek, J., Elward, K. & Gasque, P. (2003). Activation of complement in the central nervous system: roles in neurodegeneration and neuroprotection. *Ann N Y Acad Sci*, 992: 56-71.
- Wang, Y. Q., Wang, L., Zhang, M. Y., Wang, T., Bao, H. J., Liu, W. L., Dai, D. K., Zhang, L., Chang, P., Dong, W. W., et al. (2012). Necrostatin-1 suppresses autophagy and apoptosis in mice traumatic brain injury model. *Neurochem Res*, 37 (9): 1849-58.
- Varma, S., Janesko, K. L., Wisniewski, S. R., Bayir, H., Adelson, P. D., Thomas, N. J. & Kochanek, P. M. (2003). F2-isoprostane and neuron-specific enolase in cerebrospinal fluid after severe traumatic brain injury in infants and children. *J Neurotrauma*, 20 (8): 781-6.
- Weaver, S. M., Portelli, J. N., Chau, A., Cristofori, I., Moretti, L. & Grafman, J. (2012). Genetic polymorphisms and traumatic brain injury: the contribution of individual differences to recovery. *Brain Imaging Behav*.
- Veerhuis, R., Nielsen, H. M. & Tenner, A. J. (2011). Complement in the brain. *Mol Immunol*, 48 (14): 1592-603.
- Weissert, R., Wallstrom, E., Storch, M. K., Stefferl, A., Lorentzen, J., Lassmann, H., Linington, C. & Olsson, T. (1998). MHC haplotype-dependent regulation of MOG-induced EAE in rats. *J Clin Invest*, 102 (6): 1265-73.
- Wennersten, A., Holmin, S. & Mathiesen, T. (2003). Characterization of Bax and Bcl-2 in apoptosis after experimental traumatic brain injury in the rat. *Acta Neuropathol*, 105 (3): 281-8.
- West, M. J., Slomianka, L. & Gundersen, H. J. (1991). Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat Rec*, 231 (4): 482-97.
- West, M. J. (1993). New stereological methods for counting neurons. *Neurobiol Aging*, 14 (4): 275-85.
- West, M. J. (2001). Design based stereological methods for estimating the total number of objects in histological material. *Folia Morphologica*, 60 (1): 11-19.
- Whalen, M. J., Carlos, T. M., Kochanek, P. M., Clark, R. S., Heineman, S., Schiding, J. K., Franicola, D., Memarzadeh, F., Lo, W., Marion, D. W., et al. (1999). Neutrophils do not mediate blood-brain barrier permeability early after controlled cortical impact in rats. *J Neurotrauma*, 16 (7): 583-94.
- Vigh, L., Smith, R. G., Soos, J., Engelhardt, J. I., Appel, S. H. & Siklos, L. (2005). Sublethal dose of 4-hydroxynonenal reduces intracellular calcium in surviving motor neurons in vivo. *Acta Neuropathol*, 109 (6): 567-75.

- Villadangos, J. A., Schnorrer, P. & Wilson, N. S. (2005). Control of MHC class II antigen presentation in dendritic cells: a balance between creative and destructive forces. *Immunol Rev*, 207: 191-205.
- Williams, S., Raghupathi, R., MacKinnon, M. A., McIntosh, T. K., Saatman, K. E. & Graham, D. I. (2001). In situ DNA fragmentation occurs in white matter up to 12 months after head injury in man. *Acta Neuropathol*, 102 (6): 581-90.
- Wilson, M. & Montgomery, H. (2007). Impact of genetic factors on outcome from brain injury. *British Journal of Anaesthesia*, 99 (1): 43-48.
- Witgen, B. M., Lifshitz, J. & Grady, M. S. (2006). Inbred mouse strains as a tool to analyze hippocampal neuronal loss after brain injury: a stereological study. *J Neurotrauma*, 23 (9): 1320-9.
- Wong, J., Hoe, N. W., Zhiwei, F. & Ng, I. (2005). Apoptosis and traumatic brain injury. *Neurocrit Care*, 3 (2): 177-82.
- Vyas, J. M., Van der Veen, A. G. & Ploegh, H. L. (2008). The known unknowns of antigen processing and presentation. *Nat Rev Immunol*, 8 (8): 607-618.
- Yanamadala, V. & Friedlander, R. M. (2010). Complement in neuroprotection and neurodegeneration. *Trends in Molecular Medicine*, 16 (2): 69-76.
- Yang, S., Nakamura, T., Hua, Y., Keep, R. F., Younger, J. G., He, Y., Hoff, J. T. & Xi, G. (2006). The role of complement C3 in intracerebral hemorrhage-induced brain injury. *J Cereb Blood Flow Metab*, 26 (12): 1490-5.
- Yoritaka, A., Hattori, N., Uchida, K., Tanaka, M., Stadtman, E. R. & Mizuno, Y. (1996). Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. *Proc Natl Acad Sci U S A*, 93 (7): 2696-701.
- You, Z., Yang, J., Takahashi, K., Yager, P. H., Kim, H. H., Qin, T., Stahl, G. L., Ezekowitz, R. A., Carroll, M. C. & Whalen, M. J. (2007). Reduced tissue damage and improved recovery of motor function after traumatic brain injury in mice deficient in complement component C4. *J Cereb Blood Flow Metab*, 27 (12): 1954-64.
- Zauner, A., Daugherty, W. P., Bullock, M. R. & Warner, D. S. (2002). Brain oxygenation and energy metabolism: part I-biological function and pathophysiology. *Neurosurgery*, 51 (2): 289-301; discussion 302.
- Zhang, Q. G., Laird, M. D., Han, D., Nguyen, K., Scott, E., Dong, Y., Dhandapani, K. M. & Brann, D. W. (2012). Critical role of NADPH oxidase in neuronal oxidative damage and microglia activation following traumatic brain injury. *PLoS One*, 7 (4): e34504.
- Zhang, Z., Fauser, U., Artelt, M., Burnet, M. & Schluesener, H. J. (2007). FTY720 attenuates accumulation of EMAP-II+ and MHC-II+ monocytes in early lesions of rat traumatic brain injury. *J Cell Mol Med*, 11 (2): 307-14.
- Zhou, W., Xu, D., Peng, X., Zhang, Q., Jia, J. & Crutcher, K. A. (2008). Meta-analysis of APOE4 allele and outcome after traumatic brain injury. *J Neurotrauma*, 25 (4): 279-90.
- Ziebell, J. M. & Morganti-Kossmann, M. C. (2010). Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. *Neurotherapeutics*, 7 (1): 22-30.
- Zurek, J., Bartlova, L. & Fedora, M. (2011). Hyperphosphorylated neurofilament NF-H as a predictor of mortality after brain injury in children. *Brain Inj*, 25 (2): 221-6.
- Zurek, J. & Fedora, M. (2012). The usefulness of S100B, NSE, GFAP, NF-H, secretagogin and Hsp70 as a predictive biomarker of outcome in children with traumatic brain injury. *Acta Neurochir (Wien)*, 154 (1): 93-103; discussion 103.