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# Genetic networks controlling retinal injury

**Felix R. Vazquez-Chona**[1], **Amna N. Khan**[1], **Chun K. Chan**[1], **Anthony N. Moore**[2], **Pramod K. Dash**[2], **M. Rosario Hernandez**[3], **Lu Lu**[4], **Elissa J. Chesler**[5], **Kenneth F. Manly**[4], **Robert W. Williams**[4], and **Eldon E. Geisert Jr.**[1]

[1] Department of Ophthalmology, The Hamilton Eye Institute, University of Tennessee Health Science Center, Memphis, TN

[4] Center of Genomics and Bioinformatics, University of Tennessee Health Science Center, Memphis, TN

[2] Department of Neurobiology and Anatomy, The Vivian L. Smith Center for Neurologic Research, University of Texas Medical School, Houston, TX

[3] Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO

[5] Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

## Abstract

**Purpose—**The present study defines genomic loci underlying coordinate changes in gene expression following retinal injury.

**Methods—**A group of acute phase genes expressed in diverse nervous system tissues was defined by combining microarray results from injury studies from rat retina, brain, and spinal cord. Genomic loci regulating the brain expression of acute phase genes were identified using a panel of BXD recombinant inbred (RI) mouse strains. Candidate upstream regulators within a locus were defined using single nucleotide polymorphism databases and promoter motif databases.

**Results—**The acute phase response of rat retina, brain, and spinal cord was dominated by transcription factors. Three genomic loci control transcript expression of acute phase genes in brains of BXD RI mouse strains. One locus was identified on chromosome 12 and was highly correlated with the expression of classic acute phase genes. Within the locus we identified the inhibitor of DNA binding 2 (*Id2*) as a candidate upstream regulator. *Id2* was upregulated as an acute phase transcript in injury models of rat retina, brain, and spinal cord.

**Conclusions—**We defined a group of transcriptional changes associated with the retinal acute injury response. Using genetic linkage analysis of natural transcript variation, we identified regulatory loci and candidate regulators that control transcript levels of acute phase genes.

To understand the global consequences of retinal injury, several laboratories monitored the transcriptome with microarray techniques [1–5]. These changes in transcripts were analyzed to define the key regulatory events following retinal injury. In an earlier study, we defined a temporal grouping of changes related to the biochemical and cellular response to retinal injury [1]. This type of analysis, defining gene clusters that occur after injury, is not an endpoint in the analysis but a beginning. The growing number of bioinformatic resources provides an opportunity to further analyze and interpret the changes in the transcriptome after retinal injury. We can now examine the transcriptome data from injured retina by using not only various

Correspondence to: Eldon E. Geisert, Jr., Department of Ophthalmology, University of Tennessee Health Science Center, 930 Madison Avenue, Suite 731, Memphis, TN, 38163; Phone: (901) 448-7740; FAX: (901) 448-5028; egeisert@utmem.edu.

injury models [1–5] but also combining data from different regions of the central nervous system (CNS [6–9]).

Our previous analysis of rat retina identified three major transcript phases after injury: an early acute phase (within an hour), an intermediate subacute phase (within 1 to 2 days), and a late chronic phase (within 3 days to weeks [1]). The early acute phase is characterized by the transient upregulation of immediate-early genes, mainly transcription factors and activating cytokines. The intermediate subacute phase is characterized by the expression of cell-cycle and cell death genes. The late chronic phase is characterized by the expression of genes involved in neuronal and glial structural remodeling. Similar changes were described in injury models in the rat retina [2–5], brain [6,7], and spinal cord [8]. For example, the early acute genes *Fos*, *Jun*, *Egr1*, and *Nfkb1* as well as the late chronic genes *Gfap*, *A2m*, *Apoe*, and *Hsp27* display transcript changes resulting from virtually any trauma or stress throughout the CNS [1–8]. The similarities of coordinate changes in the retina, brain, and spinal cord indicate the presence of common transcriptional networks controlling the CNS response to injury.

GeneNetwork [9–12] is a powerful analytical tool to define transcriptional networks and regulators in CNS tissues. At the heart of this analysis is a unique population of mice, the recombinant inbred (RI) strains of mice. These strains were derived by inbreeding for over 20 generations the F1 crosses between C57BL/6 (B) and DBA/2J (D) mouse strains. Each BXD RI strain has a shuffled genome consisting of a unique recombination of the C57BL/6 and DBA/2J genomes, forming a segregating population of mice. These RI mouse strains, the associated microarray databases, and the genetic-analysis tools at GeneNetwork allow one to explore regulatory loci and networks built around any transcript [9–12]. The best way to think about the genetic linkage mapping is at the transcript level. If there is specific variability in transcript abundance among members of a segregating population, then it is possible to map transcript abundance to a specific chromosomal locus [9,10]. One example of this type of analysis was presented by Chesler and colleagues [9] where a group of genes involved in synaptic vesicle regulation forms a network with distinct regulatory loci. The concept of genetic analysis of transcript data is called “expression genetics” or “genetical genomics” [13]. Of interest to the vision community is the comprehensive analysis of regulatory loci controlling gene expression of the mouse brain. Both retina and brain are tissues derived from the neural tube. Thus, they share similar cell types with similar transcriptional regulation. For example, the transcription factor STAT3 plays an important role in activating cortical astrocytes [14] and retinal Müller glial cells [15]. Thus, it may be possible to use available genetic analyses of brain transcript data to define genetic networks and regulatory loci for the rat retina. To identify genomic loci and potential candidate genes controlling changes in the rat retina, we turned to our publicly available database, GeneNetwork. The GeneNetwork database was generated using RI mouse strains and a similar data set is not currently available for the rat. Since the rat is closely related to the mouse and since transcriptional expression in the normal and injured CNS is highly conserved across rodent species [16], we have chosen to use this mouse database to further analyze the changes in gene expression that occur following injury to the rat CNS.

Here we describe a method to define regulators of retinal wound healing that uses published and publicly available microarray data, transcript regulatory loci databases, and bioinformatic resources. As a starting point, we define regulators that control the expression of acute phase genes after a mechanical injury to the rat retina [1]. First, to eliminate retina-specific genes and to increase the power of our analysis, we extract a group of acute phase genes that are common to injured rat retina, brain, and spinal cord. Second, we determine the regulatory loci controlling the expression of acute phase genes in mouse brains using GeneNetwork. Third, we use computational tools to predict a candidate upstream regulator.

# METHODS

## Meta-analyses of microarray data sets

We performed a meta-analysis of previous experimental data (Table 1) to select a group of candidate genes for a common response to injury in neural tissues. We previously published CNS microarray analyses that examined the transcriptome response of injured rat retina and brain, the transcriptome of cultured human and rat astrocytes, and the regulatory loci controlling transcript expression in the mouse brain [1,6,7,9,11,17]. To complement our analysis of reactive CNS genes, we obtained publicly available microarray analyses that examined the transcriptome response of injured rat spinal cord, the transcriptome of developing mouse retina, and the transcriptome of cultured mouse neurons, mouse astrocytes, and rat microglia [8,18–21]. Table 1 makes clear the source and availability of each microarray data set. The use of microarray datasets involved three different meta-analyses. First, a set of acute phase genes commonly expressed throughout the CNS was defined by directly comparing microarray data from injured rat retina, brain, and spinal cord. For this analysis, we obtained the raw data (CEL files), determined signal values using Microarray Suite 5.0 (MAS 5.0; Affymetrix, Santa Clara, CA), transformed signals to a log scale (base 2), and normalized microarray mean intensity to 8 as described previously [1]. These transformations yielded signal intensities ranging from 1 to 18 relative units of fluorescence. Further analyses included only transcripts with medium to high abundance (that is, signals greater than 8.64) [1]. To determine significant differences, we used Student's t-test ($p<0.05$) and changes greater than 2 fold. The second meta-analysis determined if an acute phase gene was expressed in cultured astrocytes, cultured microglia, cultured neurons, and in the developing retina. An acute gene was considered present in a tissue if it displayed medium to high abundance [1]. In the third meta-analysis, we used GeneNetwork to examine transcript regulation in mouse forebrains. Transcriptome analyses with Affymetrix RG-U34A and MG-U74Av2 survey about nine and twelve thousand targets (about 30 and 40% of the rodent transcriptome, Affymetrix database, June 2005 Annotation).

## Online regulatory locus analyses

To define regulatory loci that control gene expression in mouse brain, we used GeneNetwork, which is maintained by members of our group (EJC, LL, KFM, and RWW). Details on the methods, data, and analyses are available at GeneNetwork [9–12]. Briefly, GeneNetwork is a suite of databases and analysis software that identifies regulatory loci that control transcript abundance of nearly 12,000 genes in a panel of 32 BXD RI mouse strains derived from C57BL/6 (B) and DBA/2J (D) mice, both parental strains, and the F1 hybrid (a total of 35 isogenic lines) [9–12]. Strain means for transcript abundance were generated from three to five Affymetrix U74Av2 microarrays, and each individual array was hybridized to a pool of forebrain tissue taken from three adult animals [9,11]. To maximize strain-dependent expression variance or heritability, probe-set signals were measured by weighting the perfect-match (PM) probes by their heritability [22]. Using quantitative genetic analysis and a genetic map consisting of 779 fully genotyped markers, GeneNetwork correlates the mean abundance of each transcript to genotypes at locations throughout the mouse genome. The average distance between adjacent markers is approximately 4 megabases (Mb). Here, we examined the regulation of acute phase genes in mouse brains using GeneNetwork and the weighted-by-heritability transforms (UTHSC Brain mRNA U74Av2 HWT1PM; December 2003). Variability across strains was measured using an analysis of variance (ANOVA) testing the between-strain variance compared with the total variance for 100 arrays from 35 mouse strains. The degrees of freedom for the between-group and total variance are 34 and 99. Strain-specific variation is significant ($p<0.05$) when $F_{34,99}>1.5$. Whole genome maps for all transcripts are replicated and recomputed using a variety of transforms and analytical methods in GeneNetwork, including simple and composite interval mapping, multiple-trait clustering, and

principal component analysis. We also confirmed the strength of a candidate upstream regulator using the cerebellum GeneNetwork database (SJUT Cerebellum mRNA M430 MAS5; January 2004). Since gene names may differ across species and multiple probe sets may be available in the U74Av2 microarray, we include the probe set identifier when referring to genes analyzed with GeneNetwork. We confirmed gene identity of a probe set using the Affymetrix (June 2005 Annotation) and Ensembl AffyProbe (v33) databases.

### Online bioinformatic resources

To identify candidate genes within a regulatory locus, we located genes within the appropriate genomic interval using the Genome Browser (GenomeBrowser) Mouse (May 2004 assembly). Single nucleotide polymorphisms (SNPs) were identified by comparing genome sequences in the Celera SNP (Celera Discovery Systems, CA; July 2003 Assembly), Ensembl Mouse SNPView (v33), and Entrez SNP (EntrezSNP) databases. Once the SNPs were identified, a computational approach was used to determine if the SNP within the Id2 promoter (rs4229289, for sequence variation and genomic context see Ensembl Mouse SNPView) was within a transcription factor binding site. First, we determined whether the rs4229289 SNP was located within a highly conserved region using Genome Browser Conservation tool (Mouse May 2004 Assembly). Second, we obtained a highly conserved sequence around the rs4229289 SNP from the alignment of mouse, rat, and human sequences using Genome Browser Conservation tool (May 2004 Assembly). Alignments with other species were not available. Third, we located putative transcription factor binding sites within the conserved region using the vertebrate TRANSFAC database available through MOTIF database and using the default cutoff score [23].

Several online databases provide transcript data or transcript distribution in the CNS. Gene Expression Omnibus (GEO) is a high-throughput gene expression abundance data repository, as well as a curated, online resource for gene expression data browsing, query, and retrieval. Retina Developmental Gene Expression (RetDevGE) describes the gene expression profile of thousands of genes in the developing post-natal mouse retina that were analyzed by hybridization to Affymetrix Mu74 Av2 [21]. Mouse Retina SAGE (MouseRetSAGE) Library provides serial analysis of gene expression tags or in situ hybridization images for a limited number of transcripts expressed in the developing and adult mouse retina [24]. Gene Expression Nervous System Atlas (GENSAT) maps the transcript distribution in the mouse CNS using in situ hybridization.

## RESULTS

### Acute phase genes

In our continuing efforts to understand the retinal response to injury, we concentrated on the transcriptome changes that occur within hours after retinal injury: the acute phase genes. We know that there is a sequence of changes leading to reactive gliosis and remodeling of the retina [1]. To make our analysis more robust and generalized for CNS injury, we combined our injured rat retina data with results from injured rat brain and spinal cord. In these three data sets, 30 genes demonstrated a significant change in expression level (Table 2; $p<0.05$; change>2 fold 4 h after injury). All of the common changes in the three data sets were upregulated after injury. No common downregulated transcripts were observed in injured retina, brain, and spinal cord. These acute phase genes can be expressed in a wide range of neural cells including astrocytes, microglia, and neurons (Table 2). These results suggested a generalized group of transcript changes that occurs in the CNS after injury.

The acute phase genes belong to several transcript categories including transcription, cytokines, cell adhesion, extracellular proteases, and metabolism. The major transcript group (46.6%)

was related to the regulation of gene expression including transcription factors and transcription modulators. Based on gene expression dynamics, acute phase genes displayed either transient or chronic expression changes after retinal injury (Table 2). For example, transcription factors such as *Crem*, *Egr1*, *Fos*, *Fosl1*, *Junb*, *Egr1*, *Ier3*, *Btg2*, *Atf3*, and *Nr4a1* were upregulated, with a transient surge at 4 h. Other transcription factors such as *Irf1*, *Stat3*, *Nfkb1*, *Id2*, and *Cebpd* displayed an upregulation at 4 h, followed by a smaller, but sustained, expression out to 30 days after retinal injury (Table 2).

## Regulatory locus analyses

The grouping of genes with similar transcript expression changes suggested the presence of common upstream modulators. To begin defining regulatory mechanisms controlling the retinal injury response, we tested the hypothesis that acute phase genes share common regulatory loci (Figure 1). Transcriptome-wide regulatory loci for the mouse brain were mapped previously by our group [9,11] and can be replicated and recomputed using the databases and genetic analysis tools at GeneNetwork. We used the mouse brain transcriptome and genetic analysis at GeneNetwork to define common regulatory loci for acute phase genes.

A genome-wide scan estimates the association of transcript abundance variability against genetic markers across the mouse genome. For example, the abundance variability of *Id2* was high in brains from BXD RI mouse strains ($F_{34,99}$=5.2; probe set 93013_at for *Id2* mRNA). In the genome-wide scan (Figure 1A), the correlation of *Id2* variability across the mouse genome is indicated by the likelihood ratio statistic curve (LRS; blue line). At Chromosome 12, 10–30 Mb distal to the centromere, *Id2* variation correlated highly with genetic markers *D12Mit209*, *D12Nyu7*, *Rrm2*, *D12Mit234*, and *D12Mit242* (Figure 1A, LRS=44). In a second example, *Fos* displayed variation ($F_{34,99}$=1.5; probe set 160901_at) that also correlated with genetic markers that span a region of Chromosome 12 at 10–30 Mb (Figure 1A, LRS=13). The correlation of *Fos* and *Id2* expression to a chromosomal interval on Chromosome 12 suggested that this locus contains one or more polymorphic genes that affect the levels of both transcripts.

In the group of acute phase genes, a number of transcripts had a common regulatory locus on Chromosome 12. These included *Fos*, *Nr4a1*, *Id2*, *Egr1*, *Crem*, *Junb*, *Ccl3*, and *Ptpn16*, all of which shared a strong correlation to the locus on Chromosome 12, 10–30 Mb (Figure 1B, LRS>10; probe sets 160901_at, 102371_at, 93013_at, 98579_at, 160526_at, 102362_at, 102424_at, and 104598_at, respectively). By aligning genome-wide scans, we identified a strong band of regulatory loci (Figure 1B). Most of the genes (*Fos*, *Nr4a1*, *Egr1*, *Crem*, *Junb*, *Ccl3*, and *Ptpn16*) regulated by this genomic location are found outside the regulatory locus, termed "*trans*-regulatory locus." One of the genes, *Id2*, lies within the regulatory locus (orange arrowhead, Chromosome 12 at 20.1 Mb; Figure 1). This is termed a "*cis*-regulatory locus" for *Id2*. Transcripts genetically linked to the same regulatory locus are part of a genetic network [9]. In this case, *Fos*, *Nr4a1*, *Id2*, *Egr1*, *Crem*, *Junb*, *Ccl3*, and *Ptpn16* are part of a genetic network that is controlled by the regulatory locus on Chromosome 12, 10–30 Mb. In addition to the regulatory locus on Chromosome 12, we identified two additional loci that may regulate transcript levels of acute phase genes (Figure 2). These regulatory loci were located on Chromosomes 6 and 14. In some cases, transcripts correlated to multiple regulatory loci. For example, *Fos* and *Nr4a1* were genetically linked to regulatory loci on Chromosomes 6 and 12, whereas, *Nfkb1* and *Scya2* correlated with regulatory loci on Chromosome 6 and Chromosome 14. The genetic relationships between transcript variation and genomic loci defined three genetic networks controlling the expression of acute phase genes in mouse forebrains (Figure 2). The regulators for these genetic networks are located within the regulatory loci on Chromosomes 6, 12, and 14.

### Evaluating candidate upstream regulators

Once a regulatory locus was identified, the third step in our analysis refined the genomic location responsible for the mapping to this chromosomal interval. Within each regulatory locus, a genomic element generates the variation in expression of acute phase genes. Ultimately, the genomic differences between the C57BL/6 and DBA/2J mice underlie the transcript abundance variability observed in BXD RI mouse strains. Since the BXD RI mouse strain haplotypes are finely mapped and the parental genomes are sequenced, we have thorough SNP maps for the 35 strains [9]. SNPs affecting the expression of acute phase genes may be found in a number of genomic elements including promoters, enhancers, exons, or introns. These genomic elements may affect the transcript levels of the upstream regulator. Hundreds or even thousands of SNPs may lie within a regulatory locus. Using simple rules helps to select a candidate gene. For example, a candidate gene lies within the regulatory locus, displays SNPs, and is functionally relevant to the genes correlating to the regulatory locus. Biological significance of SNPs can be queried using programs that predict whether SNPs affect known DNA binding sites or protein sequence motifs [23]. The vast biological content within online resources provides a powerful tool to identify the regulatory element controlling the expression of genes within a biological pathway, in our case the response of the transcriptome to retinal injury. We present our analysis of candidate regulators for several of the loci. We conclude with evidence for a strong candidate gene for the regulatory locus on Chromosome 12.

The regulatory locus on Chromosome 14, 35–65 Mb, modulates the transcript variability of transcription factors *Stat3*, *Nfkb1*, and *Irf1*; cytokines *Il1b* and *Scya2*; and development-related gene *Adfp* (Figure 3A, probe sets 102736_at, 103486_at, 161281_f_at, 161443_r_at, 93858_at, 98427_s_at, and 99099_at). This is a *trans*-regulatory locus for these genes, as they all lie outside this locus. A genomic element within the locus is a *trans*-regulator for these acute phase genes. Within this locus, there were 315 positional candidate genes (Figure 3B,C). A simple procedure to weigh the relevance of a positional gene is to determine whether it displays significant transcript variability in BXD RI mouse strains and whether its variability maps to its gene location: That is, does the gene display a *cis*-regulatory locus? Highly variable transcripts included *Rnase4*, *AW045965*, *M6a*, *Boct*, *Dad1*, *Ctsb*, *Cnih*, and *Ndrg2* ($F_{34,99}$>4). Of these, *Rnase4*, *AW045965*, *M6a*, *Boct*, *Boct*, *Cnih*, and *Ndrg2* linked strongly to the regulatory locus on Chromosome 14 (LRSs>17; Figure 3D; data for *Ndrg2* shown, probe set 96088_at). To further evaluate the role of these positional genes, we determined whether their self-regulatory role extended to the cerebellum of BXD RI mouse strains. *Rnase4*, *AW045965*, *M6a*, and *Ndrg2* displayed significant *cis*-regulatory loci on Chromosome 14, 35–65 Mb (LRSs>12, data not shown; probe sets 96038_at, 100073_at, 104456_at, and 161610_at). These data suggested that the regulatory role of the locus on Chromosome 14 is conserved across CNS tissues. *Rnase4*, *AW045965*, *M6a*, and *Ndrg2* are candidate upstream regulators because they display *cis*-regulatory loci.

Identifying the genes with SNPs also helps to identify the candidate upstream regulator. The sequences for *Rnase4*, *AW045965*, *M6a*, and *Ndrg2* had multiple SNPs between the C57BL/6 and DBA/2J strains. For example, *Ndrg2* had a high density of SNPs: 7 within the promoter (Celera SNPs IDs mCV23555818, mCV23556083-5, mCV23556089-91), and 25 within exons and introns (Celera SNPs IDs mCV23555752, 756-8, 762-3, 768-70, 774-6, 780-1, 788, 792-4, 804-6, 811-2, 816-7). The presence of SNPs further supported the role of *Rnase4*, *AW045965*, *M6a*, and *Ndrg2* as candidate upstream regulators. The use of transcriptome data from developing mouse retina [21,24] or injured rat retina [1] can be valuable in determining whether a candidate gene plays a role in controlling acute phase genes in the retina. In murine models of retinal development [21,24], *Ndrg2* displayed moderate transcript levels as early as postnatal day 4 and was expressed in the adult retina (Figure 3E; data available at RetDevGE [21] and MouseRetSAGE Library [24]). In contrast, *Rnase4*, *AW045965*, and *M6a* were absent

from birth through adult retinal stages (data available at RetDevGE). Using a suite of computational tools and transcriptome data from CNS gene regulation and development, we defined *Ndrg2* (N-myc downstream regulated gene 2) as a positional candidate that displayed a *cis*-regulatory locus in the mouse brain and cerebellum and that has a high SNP density. At the present time, *Ndrg2* is the best candidate gene for the regulatory locus on Chromosome 14 and for potentially being a regulator of retinal acute genes. *Ndrg2* displays a conserved self-regulatory role in mouse brain and cerebellum. It is expressed in the developing mouse retina during the critical time of cell proliferation and cell specification. It is expressed in the developing mouse retina during the critical time of cell proliferation and cell specification. It displays persistent transcript expression in the adult mouse retina.

The regulatory locus on Chromosome 6 at 105–132 Mb also controls the expression of acute phase genes such as *Fosl1*, *Scya2*, *Nr4a1*, *Fos*, *Nfkb1*, *Icam1*, and *Timp1* (Figure 4A; probe sets 102371_at, 102736_at, 160901_at, 99835_at, 101141_at, 98427_s_at, and 101464_at). Within the locus, there were 315 positional genes (Figure 4B). Using the computational and comparative techniques described above, *Ccnd2* (Cyclin D2) best met the criteria for a candidate upstream regulator. *Ccnd2* displayed significant transcript abundance variability ($F_{34,99}$=3.8, Figure 4C, probe set 97504_at) and a strong *cis*-regulatory locus on Chromosome 6 (LRS=29, Figure 4D) in brains from BXD RI mouse strains. We also observed a strong *cis*-regulatory locus for *Ccnd2* in the cerebellum (LRS=15; probe set 1416122_at_A; data not shown). Multiple SNPs spanned the transcribed region of *Ccnd2* (Celera SNP IDs mCV24153285, 558, 560, 570, 580-2, 592-4, 615-6). These data suggested that *Ccnd2* might control the expression of classic acute genes in the CNS of BXD RI mouse strains. In the mouse retina, *Ccnd2* is expressed during development and adult stages (data available at Mouse Retina SAGE Library and GENSAT Image 14340). Moreover, *Ccnd2* displayed chronic upregulation in the injured rat retina (data available at GEO data set GSE1001, probe set rc_AA899106_at [1]). Several data defined *Ccnd2* as the best current candidate gene for the regulatory locus on Chromosome 6 and for potentially being a regulator of retinal reactive genes. It displays conserved self-regulatory role in mouse brain and cerebellum. It is expressed in the developing mouse retina during the critical time of cell proliferation and cell specification. It is expressed in the developing mouse retina during the critical time of cell proliferation and cell specification. It displays chronic upregulation in the injured rat retina.

For the network defined by the regulatory locus on Chromosome 12 (Figure 5A, probe sets 93013_at, 161802_i_at, 161716_at, 160526_s_at, 104598_at, 102424_at, 102371_at, and 102362_i_at), the *Inhibitor of DNA binding 2* (*Id2*) is the best current candidate upstream regulator. First, the positional candidate *Id2* (Figure 5B; probe set 93013_at) showed significant variability and strong *cis*-regulatory locus in brains from BXD RI mouse strains ($F_{34,99}$=5.2, Figure 5C; and LRS=44; Figure 5D). In the cerebellum, we also observed a strong *cis*-regulatory locus for *Id2* (LRS=13; probe set 1453596_at_A; data not shown). Second, while several *cis*-regulated genes were located within the regulatory locus on Chromosome 12 (for example, *Lpin1* [probe set 98892_at]), only *Id2* was upregulated in the rat retina, brain, and spinal cord after injury (Table 2). In the injured rat retina, *Id2* also displayed a chronic upregulation even after 30 days postinjury (Table 2; data available at GEO data set GSE1001, probe set rc_AI230256_at). *Id2* was also highly expressed during mouse retinal development at the time of high levels of cell proliferation and remained present in the adult mouse retina (data available at Retina Developmental Gene Expression and Mouse Retina SAGE Library). Third, we located one SNP for *Id2* within the promoter and four SNPs within the second intron (Figure 5D; Ensembl gene ID ENSMUSG00000020644). An SNP within the promoter region of *Id2* may alter the ability of a transcription factor to modulate *Id2* transcription.

We used in silico promoter analysis to define the transcription factor binding site affected by the SNP (Ensembl SNPView ID rs4229289 and Celera SNP ID mC22302957). Located 320

base pairs upstream of the starting codon, the SNP consisted of an adenine-guanine (A/G) substitution between the DBA/2J and C57BL/6. The SNP was also located in a highly conserved region across mouse, rat, and human orthologs (Figure 5F). Using the MOTIF database, we determined that the SNP sits next to a CCAAT binding site (TRANSFAC ID M00254), a likely binding site for nuclear transcription factor (NF)-Y (TRANSFAC ID M00185). Based on the TRANSFAC 5.0 database available through the MOTIF website, NF-Y binds to the CCAAT motif when the adjacent 3′-nucleotide is either G or C as seen in the C57BL/6, rat, and human sequences for *Id2* (Figure 5F). The nucleotide substitution of an A for a G/C may account for the lower *Id2* levels seen in the DBA/2J and in all the BXD strains carrying the DBA/2J haplotype. Several lines of evidence indicate that *Id2* is a good candidate gene for the regulatory locus on Chromosome 12. *Id2* is conserved and has a self-regulatory role in mouse brain and cerebellum. It is expressed in the developing retina during the critical time of cell proliferation and cell specification. It is upregulated immediately following injury to rat retina, brain, and spinal cord.

## DISCUSSION

Discovering mechanisms controlling retinal wound healing from gene expression data is a promising and challenging task. The method of extracting genetic networks and gene regulation presented here involves (1) highlighting robust injury genes in multiple models of CNS trauma, (2) defining regulatory loci using genetic analysis of transcript data (that is, expression genetics [13]) from CNS tissues, and (3) predicting candidate regulators using bioinformatic resources that are available online. Using this approach, we defined a set of acute phase genes that is commonly expressed in the retina, brain, and spinal cord after traumatic injury. Our expression genetic analyses revealed that regulatory loci on Chromosomes 6, 12, and 14 control the expression of acute phase genes in brains from BXD RI mouse strains. With the help of an array of online bioinformatic tools, we identified three genetic networks and candidate upstream regulators: *Ndrg2*, *Ccnd2*, and *Id2*.

Surprisingly, the changes controlled by these three loci are present in a variety of different CNS tissues: retina, brain, and spinal cord. This begs the question, are the networks controlled by the loci on Chromosomes 6, 12, and 14 unique to the CNS? If one examines normal tissues, these regulatory loci appear to be unique to the CNS. For example, expression genetics of hematopoietic stem cells from BXD RI mouse strains showed completely different sets of regulatory loci [10]. This is not surprising given the difference in expression patterns between CNS tissues and hematopoietic stem cells [9,10]. The same is true of the liver where Schadt and colleagues [25] identified a different set of regulatory loci that did not correlate with our observations in the CNS. Although the specificity of expression-related regulatory loci in CNS, hematopoietic stem cells, and liver are different in normal resting conditions, we cannot exclude the possibility that some regulatory mechanism are similar following trauma. Based upon our current data, the loci identified at chromosomes 6, 12, and 14 appear specific to the CNS.

Our method complements approaches that identify modules of co-regulated genes, shared *cis*-regulatory motifs, and transcriptional factors binding to *cis*-regulatory motifs [26]. In reality, transcriptome changes during the wound-healing response are controlled by a much more complex network [27–30]. Our method holds the potential for identifying members of genetic networks and regulators that may include gene products involved in mRNA stability, heterochromatin remodeling, transcriptional repression, and other regulatory mechanisms [9–12,25]. Indeed, even indirect modifiers of the transcriptional response to injury that are not transcription factors can be identified using a genetic approach. Our analysis suggests, for example, that the expression of acute phase genes is genetically linked to the transcript

expression of three non-transcription factors including the hydrolase NDRG2, the cyclin CCND2, and the dominant negative helix-loop-helix protein ID2.

Key to defining modulators of regulatory networks in BXD RI mouse strains is the genetic differences between parental strains. Genetic differences between C57BL/6 and DBA/2J strains provide a powerful system to study regulators and their targets, but this approach is limited to the detection of polymorphisms between these strains. For some genes believed to be involved in the response of the CNS to injury there are no genetic differences between the BXD parental strains. For example, STAT3 (a potent activator of acute phase genes such as *Fos* [31,32]) has no SNPs in the coding or regulatory regions when the sequence of *Stat3* of C57BL/6 strain is compared to that of the DBA/2J strain. Consequently, expression genetics of BXD RI mouse strains revealed no genetic linkage between the *Stat3* locus and genes known to be associated with its regulation such as *Fos*. To provide a fuller examination of the networks controlling retinal wound healing the BXD RI strains can be supplemented by studying additional strains. Other RI strains of mice can be equally valuable. The BXA and AXB RI strains were produced from crosses of the C57BL/6 (B) and A/J (A) mouse strains. Genetic differences between these two strains can be used to map different regulatory loci. One prime example of the use of the AXB RI strains comes from a study of horizontal cell density. A 2 fold difference in horizontal cell density was observed between C57BL/6 and A/J mouse strains [33]. The genetic backgrounds of the A/J mouse strains and RI strains derived from the C57BL/6 and A/J mouse strains make it possible to map genetic loci controlling horizontal cell number and potentially the response of horizontal cells to retinal injury. Undoubtedly as more genetically diverse sets of strains become available, transcriptome-wide analysis combined with genetic analysis will yield additional regulatory loci. This type of analysis and the development of additional expanded RI strains of mice hold great promise in the search for genetic networks controlling complex biological processes such as wound healing in the retina and CNS.

Typically, a candidate upstream modulator within regulatory loci will display (1) high transcript variation that maps onto its transcript location, that is, a *cis*-regulatory locus, (2) polymorphisms between parental strains, and (3) a function related to CNS disease or expression changes after CNS injury. *Ndrg2*, *Ccnd2*, and *Id2* met the candidate gene criteria for the regulatory loci on Chromosomes 14, 6, and 12, respectively. N-myc downstream regulated gene 2 (NDRG2) is found at high protein levels in neurogenic regions of the adult rat brain. In these regions, NDRG2 localizes to GFAP-positive astrocytes or radial glia [34]. In humans, downregulation of *Ndrg2* is associated with glioblastoma [35]. High levels of NDRG2 are often associated with senile plaques of Alzheimer's patients and cellular processes of dystrophic neurons [36]. Our meta-analyses of *Ndrg2* using transcript data from developing mouse retina showed that *Ndrg2* was expressed during the time of cell proliferation and cell specification. These data suggest that NDRG2 may play a key role in the proliferation of glia cells and the degeneration of neurons.

A second likely upstream candidate of CNS injury is cyclin D2 (CCND2). Cyclin D2 phosphorylates the Rb protein of the RB-E2F complex. Cyclin D2 binding to the Rb protein allows the transcription factor E2F to enter the nucleus and stimulate proliferation. During the development of the cerebellum, cyclin D2 is a marker of proliferating granule cell precursors. Granule neurons are the principal neuronal component of cerebellum, and mice deficient in cyclin D2 show decreases in the number of granule cells and stellate interneurons [37,38]. Our meta-analyses of *Ccnd2* also indicated that *Ccnd2* was moderately expressed in cultured astrocytes from the cortex and optic nerve, in the mouse developing retina, and injured rat retina. The enhanced expression of cyclin D2 in the retina following trauma may contribute to reactive responses such as cellular proliferation.

The third and most promising upstream candidate of CNS injury is the inhibitor of DNA binding 2 (ID2). Our analyses suggest that changes in *Id2* expression were a common theme in many reactive CNS tissues. Based on our meta-analyses of microarray data, *Id2* was upregulated in rat retina, brain, and spinal cord after mechanical injury. In rat models of glaucoma [3], the dystrophic retina also upregulates *Id2* (personal communication, Drs. Farid Ahmed and Stanislav Tomarev, NEI, NIH). We have confirmed upregulation of *Id2* transcript in mouse retina after a mechanical and toxic injury (data not shown). Optic nerve head astrocytes from patients suffering glaucoma display higher *Id2* transcript levels than astrocytes from non-glaucomatous patients [39]. The role of ID2 as a positive regulator of cell-cycle progression [40–42] is consistent with the proliferative response that occurs in retinal injury. A potential source for dividing cells in the inner nuclear layer is the Müller cells [43–45]. When Müller cells enter the cell cycle, they decrease the expression of cell cycle inhibitors, including the cyclin-dependent kinase inhibitor p27/Kip1 [43]. ID2 is known to negatively regulate the transcription of cell cycle inhibitor genes, including p15, p16, and p21 [40]. ID2 downregulates expression of cell cycle inhibitors by preventing the interaction of basic helix-loop-helix (bHLH) transcription factors that promote the transcription of cell cycle inhibitor genes [40]. Alternatively, ID2 may stimulate proliferation by binding to the unphosphorylated Rb protein, allowing the release of the transcription factor E2F. E2F in turn activates genes involved in the G1 to S transition and hence proliferation [40,41]. Re-entry into the cell cycle may also indicate apoptotic activity [46]. ID2 is known to play a role in neuronal apoptosis [47]. Cerebellar granule neurons upregulate transcript and protein levels of ID2 during the onset of apoptosis [47]. The expression of *Id2* antisense RNA in neurons protects from apoptosis. Together these data suggest that the increase in ID2 may stimulate cells of the INL into the G1-S transition of the cell cycle either for proliferation or apoptosis. We are currently using the genetic background offered by the BXD RI mouse strains to determine the role of the Chromosome 12 locus and ID2 in retinal wound healing. We have confirmed the variability of ID2 at the transcript and protein level in retinas of BXD RI mouse strains (data not shown). We are using high and low Id2 expressers to see the effect of Id2 dose effect on wound healing events following retinal trauma.

The novel combination of microarray analysis, expression genetics, and bioinformatics provides a new and powerful approach to defining regulatory elements in the genome. Using this approach, we were able to generate specific, testable hypotheses defining pathways that regulate proliferative and reactive responses in the retina and elsewhere in the CNS. As more diverse gene expression data sets become available, it is our belief that comparison of gene expression and regulation in different biological contexts will help identify the regulatory elements controlling the reactive response in the retina.

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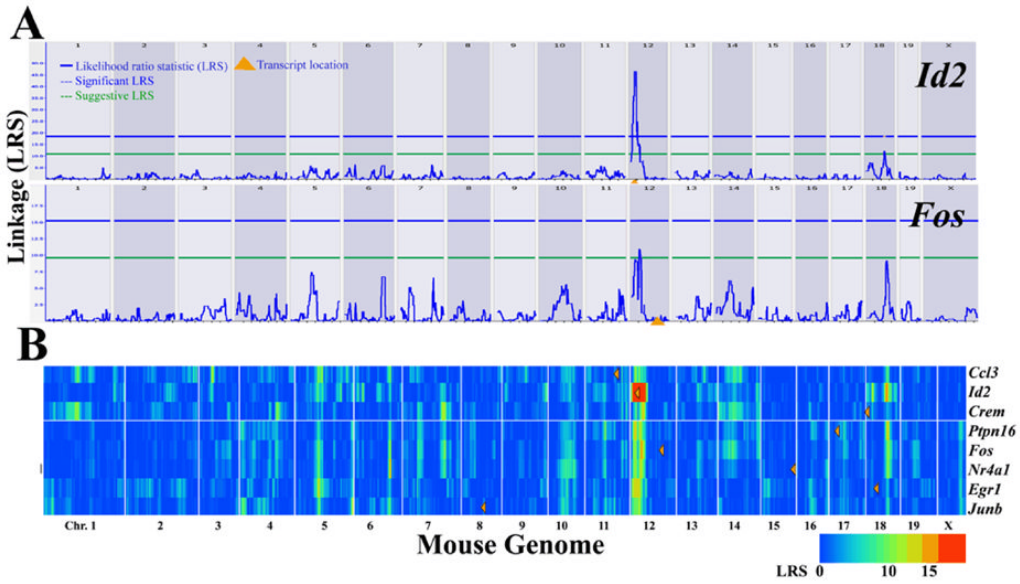


**Figure 1.**

Acute phase genes are modulated by distinct regulatory loci. We used the genetic analysis of transcript expression at GeneNetwork to define genomic loci that control transcript abundance variability in mouse brains. At the heart of this analysis is a unique population of mice, the recombinant inbred (RI) strains of mice. One group of mice were derived by inbreeding for over 20 generations the F1 crosses between C57BL/6 and DBA/2J mouse strains. Each BXD RI strain has a shuffled genome consisting of a unique recombination of the C57BL/6 and DBA/2J genomes, forming a segregating population of mice. These RI mouse strains, the associated microarray databases, and the genetic-analysis tools at GeneNetwork allow one to explore regulatory loci and networks built around any transcript [9–12]. The best way to think about the genetic linkage mapping is at the transcript level. If there is specific variability in transcript abundance among members of a segregating population, then it is possible to map transcript abundance to a specific chromosomal locus. Genetic linkage maps show that a group of acute phase genes is controlled by a regulatory locus on Chromosome 12. **A**: Individual genome-wide maps for *Id2* and *Fos* display linkage of transcript variability (y-axis) to loci across the mouse genome (x-axis). Genetic analysis of transcript data from brain tissue was determined in a panel of BXD recombinant inbred (RI) mouse strains [9,11]. Maps were generated by linking transcript variability against 779 genetic markers that are interspaced along the mouse genome by an average of 4 Megabase pairs (Mb). The alternating white and blue columns represent chromosomes. The linkage between transcript variation and genetic differences at a particular genetic locus is measured in terms of likelihood ratio statistic (LRS; solid blue line). Dashed horizontal lines mark transcript-specific significance thresholds for genome-wide $p<0.05$ (significant, blue) and genome-wide $p<0.63$ (suggestive, green). Orange triangle indicates gene location. **B**: Multiple parallel genome-wide maps display linkage using color hues with yellow and red bands representing suggestive and significant LRS. A common regulatory locus on Chromosome 12 is identified for selected acute phase genes. Within this locus, there is a genomic element modulating the expression of acute phase genes such as *Fos*, *Junb*, and *Egr1*. Individual and multiple genome-wide maps were generated using the tools at GeneNetwork. Orange triangles indicate gene location. Linkage maps were generated using the Interval Mapping and Cluster Tree tools at GeneNetwork (UTHSC Brain mRNA U74Av2 HWT1PM; December 2003).

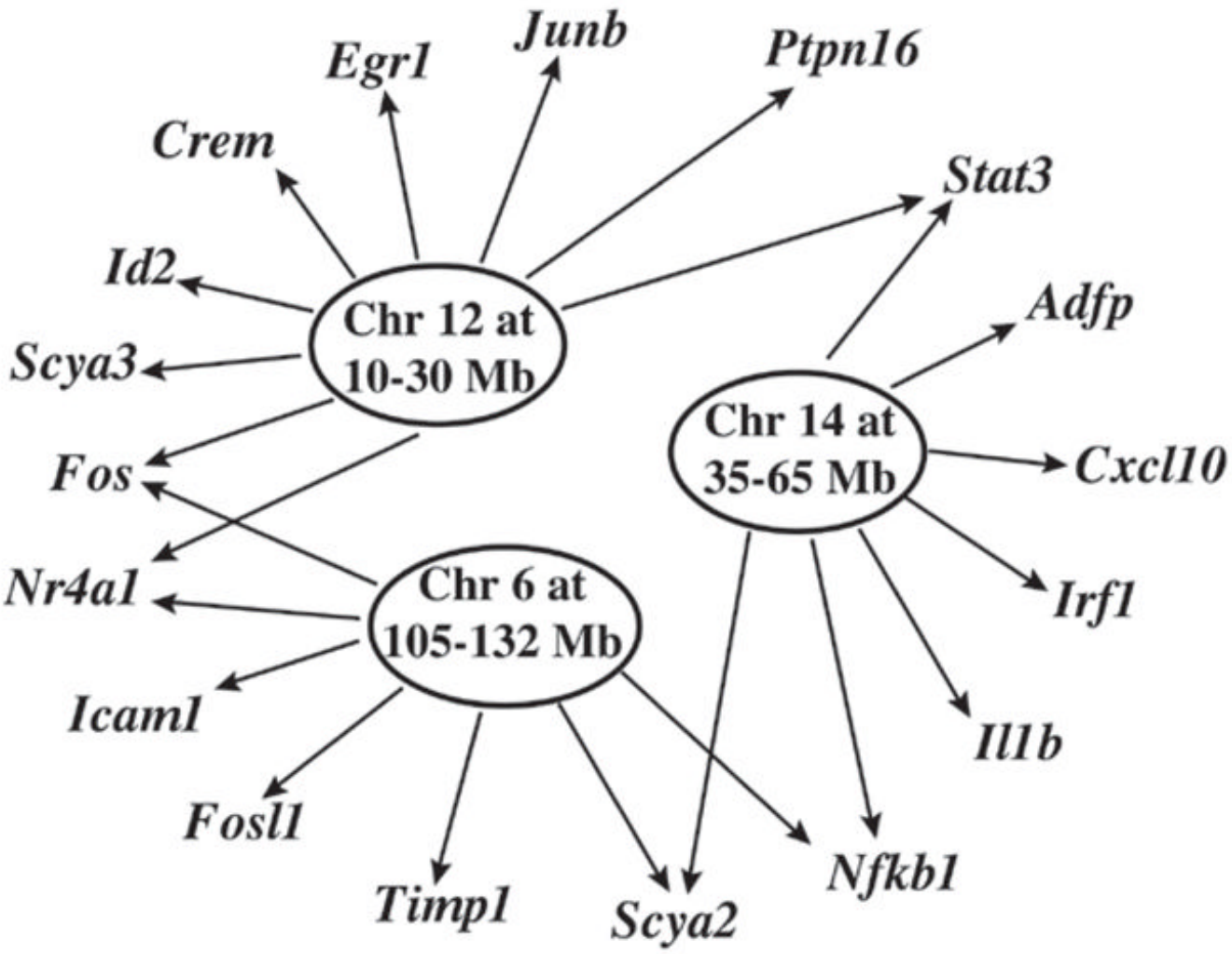


**Figure 2.**

Genetic networks controlling acute phase transcripts. Genetic networks were derived from transcripts sharing regulatory loci in mouse brains. As shown in Figure 1, acute phase transcripts *Fos*, *Egr1*, *Nr4a1*, *Junb*, *Stat3*, *Id2*, *Crem*, *Ptpn16*, and *Scya3* are controlled by a genomic locus on Chromosome 12. We also showed in Table 2 that these transcripts are part of a common phase response to injury in retina, brain, and spinal cord. Transcripts genetically linked to the same regulatory loci and highly regulated in injured CNS are hypothesized to be part of a genetic network. In this diagram, lines connecting specific genes to loci represent a correlation between transcript expression and the regulatory locus. The diagram also illustrates two networks controlled by genomic loci on Chromosomes 6 and 14.

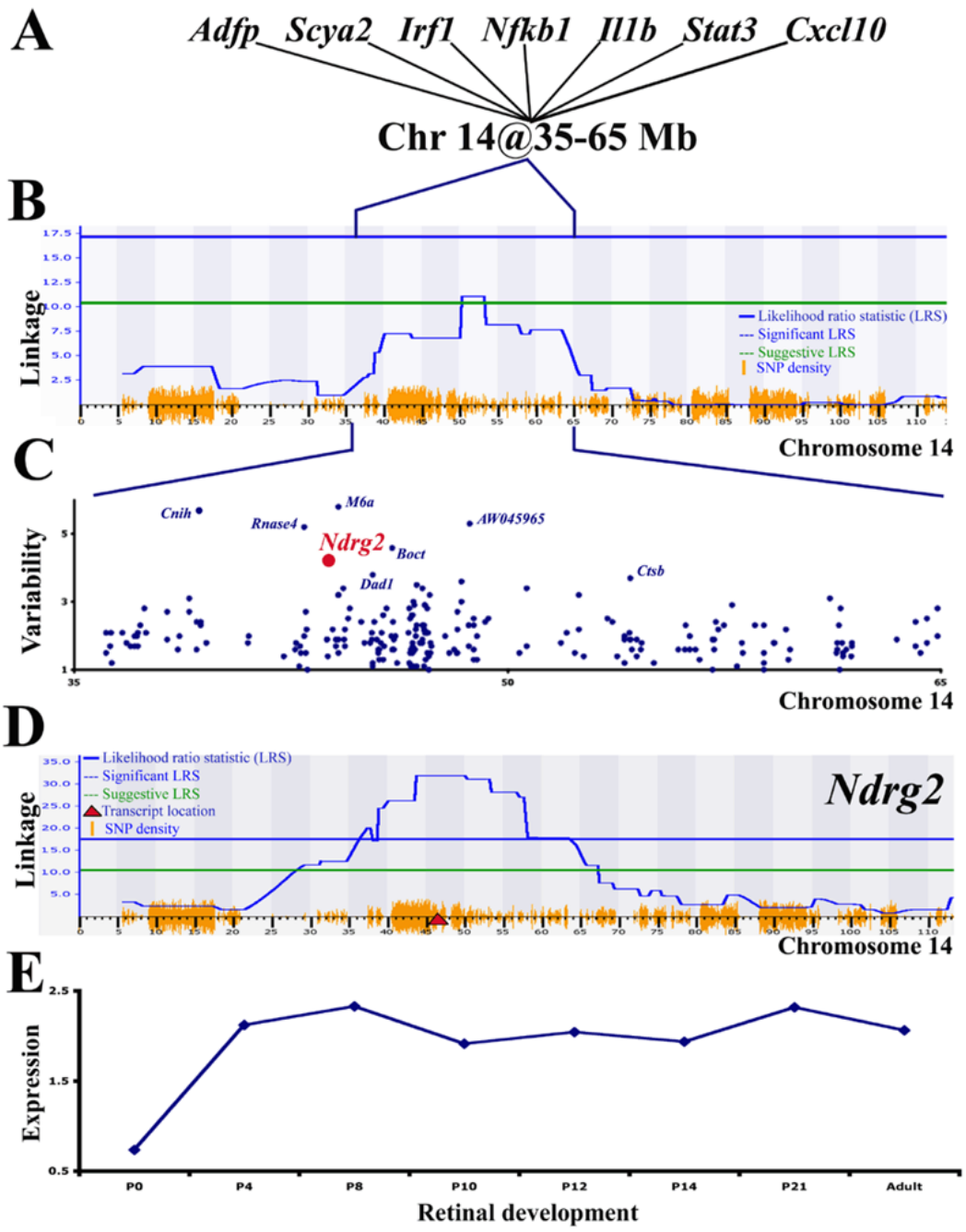


**Figure 3.**

Evaluating candidate genes within Chromosome 14 locus. **A**: The transcript variability of *Adfp*, *Scya2*, *Irf1*, *Nfkb1*, *Il1b*, *Stat3*, and *Cxcl10* in brains from BXD RI mouse strains is genetically linked to a regulatory locus on Chromosome 14. This network was generated by comparing genome-wide scans that measure the linkage of transcript variability across the mouse genome, as described in Figure 1. These transcripts are also upregulated as acute phase transcripts in retina, brain and spinal cord (Table 2). Transcripts genetically linked to the same regulatory loci and highly regulated in injured CNS are hypothesized to be part of a genetic network. **B**: The combined genome-wide map shows that their transcript expression has a strong genetic linkage to a locus within Chromosome 14. The genome wide scan, zoomed at

Chromosome 14, shows the genetic linkage (y-axis, likelihood ratio statistic [LRS]) across the chromosome (x-axis, Megabase pairs [Mb]). The locus (35 to 65 Mb) includes 315 genes, some of which have a high or low single nucleotide polymorphism (SNP) density. SNP density is denoted by the height of the orange lines on the x-axis. Several rules may help in selecting candidate gene responsible for this regulatory locus. The first rule is there have to be SNPs present within the coding or regulatory region of the gene of interest. As a first approximation we used SNP density that is displayed at the bottom of panel B and D. The second rule is a high degree of transcript abundance variability in the BXD RI strains (illustrated in **C**). **C**: Transcript abundance variability in normal forebrains of BXD RI mouse strains is due to genetic polymorphisms between the parental C57BL/6 and DBA/2J mouse strains. The graph illustrates transcript abundance variability (y-axis) for genes (dots) within the 35 to 65 Mb interval of Chromosome 14 (x-axis). For a transcript to be mapped its abundance must vary across the BXD RI mouse strains. The higher the variation in transcript abundance the more likely that the gene is a candidate. Furthermore we expect that the variability is due to a polymorphism in the candidate gene, suggesting that the polymorphic gene is modulating its own expression level (that is, a "*cis*-regulatory locus"). We measured transcript variability using an analysis of variance (ANOVA) that tests the between-strain variance compared with the total variance for 100 arrays from 35 mouse strains. The degrees of freedom for the between-group and total variance are 34 and 99. Strain-specific variation is significant ($p<0.05$) when $F_{34,99}>1.5$. In the graph, each dot represents a gene with its variability measured by the ANOVA F-statistic (y-axis) and its genomic location within Chromosome 14 described in Mb. *Rnase4*, *AW045965*, *M6a*, *Boct*, *Dad1*, *Ctsb*, *Cnih*, and *Ndrg2* are polymorphic genes that displayed significant transcript variability and *cis*-regulatory loci in normal forebrains of BXD mouse strains. **D**: The third criterion identifies the genes that display self regulation across CNS tissues. For example, the transcript variability of *Ndrg2* (red circle in **C**) displayed a *cis*-regulatory locus in brain (LRS=38, probe set 96088_at) and cerebellum (LRS=13, probe set 1448154_at_A) of BXD RI mouse strains. Here we illustrate linkage of *Ndrg2* transcript variability in BXD RI mouse strains across Chromosome 14. Red triangle indicates gene location. *Rnase4*, *AW045965*, and *M6a* also displayed *cis*-regulatory loci in brain and cerebellum (data available at GeneNetwork). **E**: The fourth criterion deals with the biology of the system. Meta-analyses of transcript levels during retinal development and retinal injury can help determine if a candidate gene is a potential regulator in the retina. For example in murine models of retinal development, *Ndrg2* displayed moderate transcript levels as early as postnatal day 4 and was highly expressed in the adult retina. In contrast, *Rnase4*, *AW045965*, and *M6a* were absent from birth through adult stages. Normalized expression data for *Ndrg2* (y-axis) during murine retinal development (x-axis) is available at RetDevGE [21].

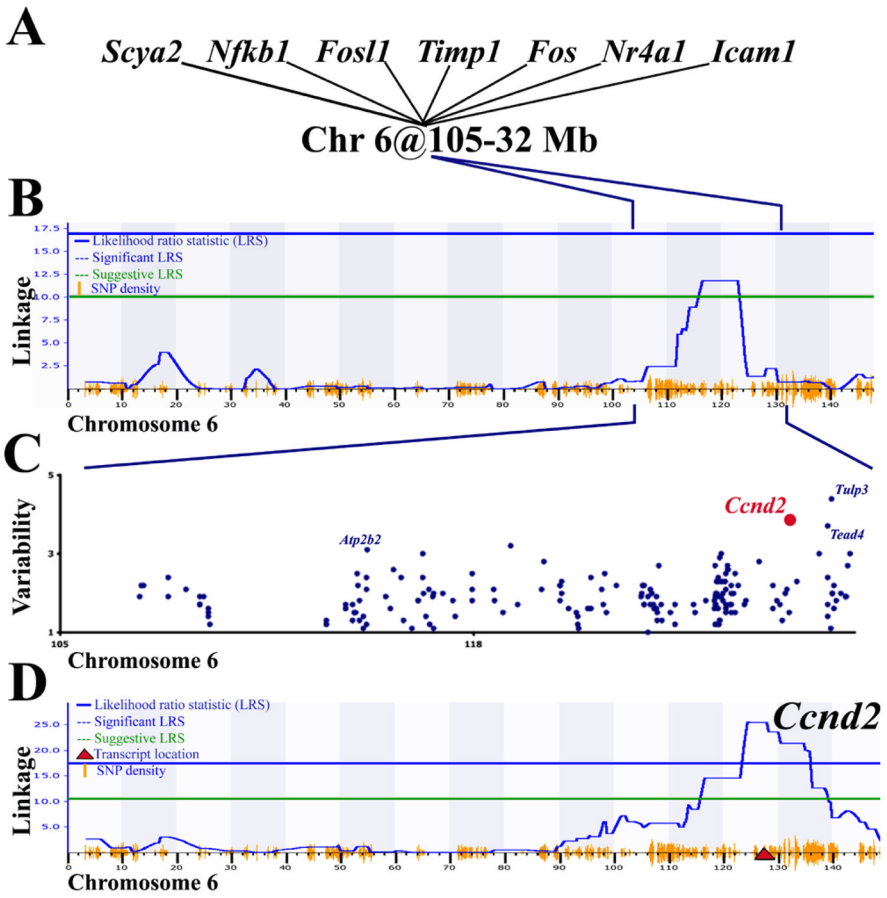


**Figure 4.**

Evaluating candidate genes within Chromosome 6 locus. **A**: The transcript variability of *Scya2*, *Nfkb1*, *Fosl1*, *Timp1*, *Fos*, *Nr4a1*, and *Icam1* in brains from BXD RI mouse strains is genetically linked to the regulatory locus on Chromosome 6. This network was generated by comparing genome-wide scans that measure the linkage of transcript variability across the mouse genome, as described in Figure 1. These transcripts are also upregulated as acute phase transcripts in retina, brain and spinal cord (Table 2). Transcripts genetically linked to the same regulatory loci and highly regulated in injured CNS are hypothesized to be part of a genetic network. **B**: The combined genome-wide map shows that their transcript expression has a strong genetic linkage to a locus within Chromosome 6, 105 to 132 Mb. The genome wide scan, zoomed at Chromosome 6, shows the genetic linkage (y-axis, likelihood ratio statistic [LRS]) across the chromosome (x-axis, Megabase pairs [Mb]). This locus includes over 200 positional genes, some of which have a high or low single nucleotide polymorphism (SNP) density. SNP density is denoted by the height of the orange lines on the x-axis. Several rules may help in selecting candidate gene responsible for this regulatory locus. The first rule is there have to be SNPs present within the coding or regulatory region of the gene of interest. As a first approximation we used SNP density that is displayed at the bottom of panel B and D. The second rule is a high degree of transcript abundance variability in the BXD RI strains (illustrated in **C**). **C**: Transcript abundance variability in normal forebrains of BXD RI mouse strains is due to genetic polymorphisms between the parental C57BL/6 and DBA/2J mouse strains. The graph illustrates transcript abundance variability (y-axis) for genes (dots) within the 105 to 132 Mb interval of Chromosome 6 (x-axis). The higher the variation in transcript abundance the more likely that the gene is a candidate. Furthermore we expect that the variability is due to a

polymorphism in the candidate gene, suggesting that the polymorphic gene is modulating its own expression level (that is, a "*cis*-regulatory locus"). We measured transcript variability using an analysis of variance (ANOVA) that tests the between-strain variance compared with the total variance for 100 arrays from 35 mouse strains. The degrees of freedom for the between-group and total variance are 34 and 99. Strain-specific variation is significant ($p<0.05$) when $F_{34,99}>1.5$. In the graph, each dot represents a gene with its variability measured by the ANOVA F-statistic (y-axis) and its genomic location within Chromosome 6 described in Mb. *Tulp3*, *Ccnd2*, *Tead4*, and *Atp2b2* are polymorphic genes that displayed significant transcript variability and *cis*-regulatory loci in normal forebrains of BXD mouse strains. **D**: The third criterion identifies the genes that display self regulation across CNS tissues. For example, the transcript variability of Ccnd2 (red circle in C) displays a strong *cis*-regulatory locus in the forebrain (LRS=25, probe set 97504_at) and cerebellum (LRS=14, probe set probe set 1416122_at_A) of BXD RI mouse strains. Here we illustrate linkage of *Ccnd2* transcript variability in BXD RI mouse strains across Chromosome 6. Red triangle indicates gene location. Atp2b2, Tulp3, and Tead4 also displayed *cis*-regulatory loci in brain and cerebellum (data available at GeneNetwork). The role of a candidate gene as a modulator of the Chromosome 6 and wound healing is further strengthen if its transcript is differentially expressed in injured CNS. *Ccnd2* displays increased transcript levels in injured rat retina (data available at GEO data set GSE1001, probe set rc_AA899106_at) [1].

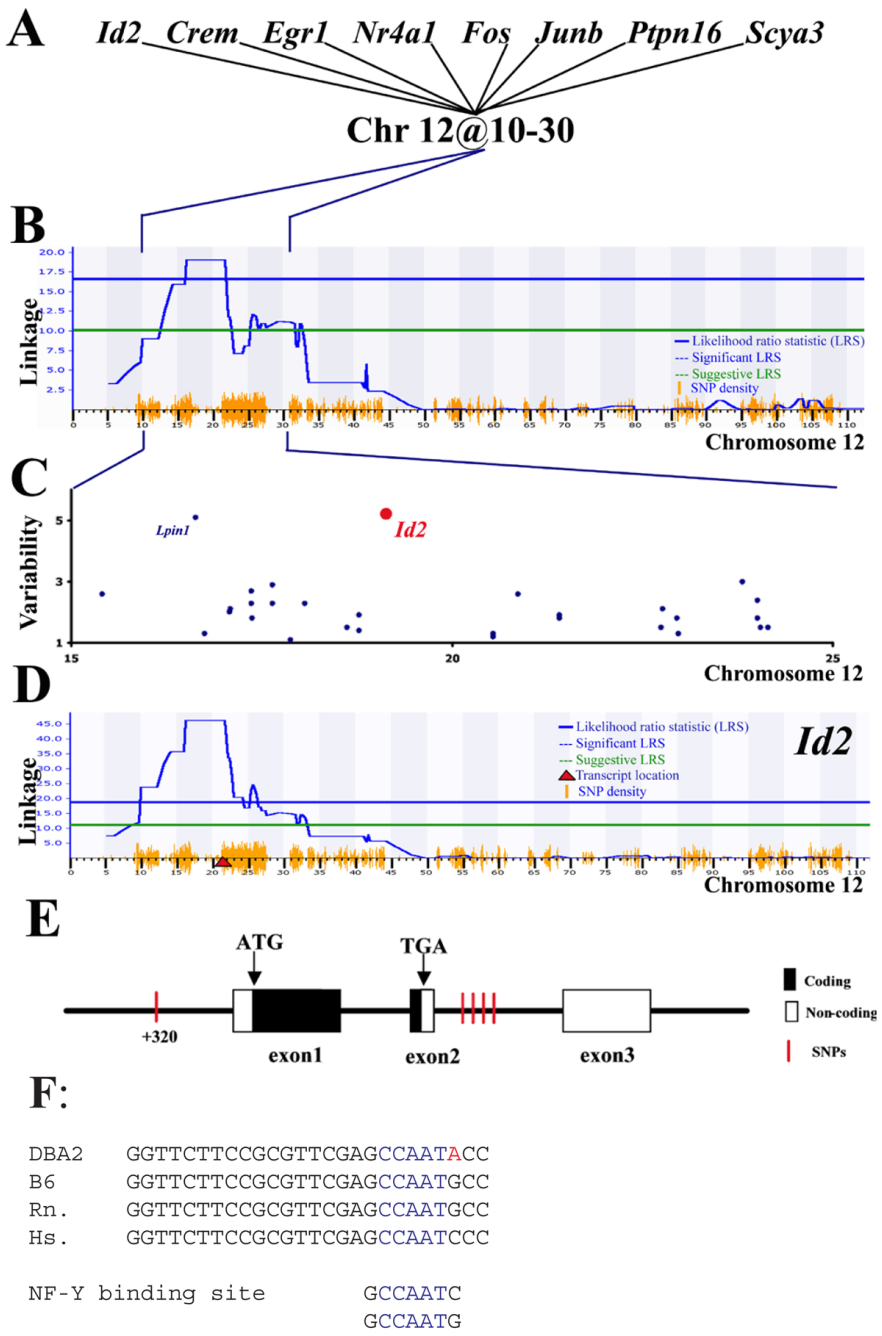


**Figure 5.**

Evaluating candidate genes within Chromosome 12 locus. **A**: The transcript variability of *Id2*, *Crem*, *Egr1*, *Fos*, *Nr4a1*, *Junb*, *Ptpn16*, and *Icam1* in brains from BXD RI mouse strains is genetically linked to the regulatory locus on Chromosome 12. This network was generated by comparing genome-wide scans that measure the linkage of transcript variability across the mouse genome, as described in Figure 1. These transcripts are also upregulated as acute phase transcripts in retina, brain and spinal cord (Table 2). Transcripts genetically linked to the same regulatory loci and highly regulated in injured CNS are hypothesized to be part of a genetic network. **B**: The combined genome-wide map shows that their transcript expression has a strong genetic linkage to a locus within Chromosome 12, 10 to 30 Mb. The genome wide scan,

zoomed at Chromosome 12, shows the genetic linkage (y-axis, likelihood ratio statistic [LRS]) across the chromosome (x-axis, Megabase pairs [Mb]). This locus includes over 60 positional genes, some of which have a high or low single nucleotide polymorphism (SNP) density. SNP density is denoted by the height of the orange lines on the x-axis. Several rules may help in selecting candidate gene responsible for this regulatory locus. The first rule is there have to be SNPs present within the coding or regulatory region of the gene of interest. As a first approximation we used SNP density that is displayed at the bottom of panel B and D. The second rule is a high degree of transcript abundance variability in the BXD RI strains (illustrated in **C**). **C**: Transcript abundance variability in normal forebrains of BXD RI mouse strains is due to genetic polymorphisms between the parental C57BL/6 and DBA/2J mouse strains. The graph illustrates transcript abundance variability (y-axis) for genes (dots) within the 10 to 30 Mb interval of Chromosome 12 (x-axis). For a transcript to be mapped its abundance must vary across the BXD RI mouse strains. The higher the variation in transcript abundance the more likely that the gene is a candidate. Furthermore we expect that the variability is due to a polymorphism in the candidate gene, suggesting that the polymorphic gene is modulating its own expression level (that is, a "*cis*-regulatory locus"). We measured transcript variability using an analysis of variance (ANOVA) that tests the between-strain variance compared with the total variance for 100 arrays from 35 mouse strains. The degrees of freedom for the between-group and total variance are 34 and 99. Strain-specific variation is significant ($p<0.05$) when $F_{34,99}>1.5$. In the graph, each dot represents a gene with its variability measured by the ANOVA F-statistic (y-axis) and its genomic location within Chromosome 12 described in Mb. *Lpin1* and *Id2* are polymorphic genes that displayed significant transcript variability and *cis*-regulatory loci in normal forebrains of BXD mouse strains. **D**: The third criterion identifies the genes that display self regulation across CNS tissues. For example, the transcript variability of Id2 (red circle in C) displayed a *cis*-regulatory locus in the brain (LRS=44, probe set 93013_at) and cerebellum (LRS=14; 1453596_at_A) of BXD RI mouse strains. Here we illustrate linkage of Id2 transcript variability in BXD RI mouse strains across Chromosome 12. Red triangle indicates gene location. *Lpin1* also displayed *cis*-regulatory loci in brain and cerebellum (data available at GeneNetwork). **E**: The structure of the Id2 gene illustrates a SNP at the promoter region and four SNPs within the second intron. **F**: The SNP within the promoter region (Ensembl SNPView ID rs4229289 and Celera SNP ID mC22302957) is located within a highly conserved region and is adjacent to a nuclear transcription factor Y (NF-Y) binding site (TRANSFAC ID M00185). Gene structure was obtained from Ensembl and GenomeBrowser. The TRANSFAC 5.0 database was accessed through the MOTIF website. Highly conserved regions were defined using the GenomeBrowser Conservation tool (Mouse May 2004 Assembly).

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**Table 1**

Data used to define acute phase genes

| Tissue | Reactive response inducer | Species | Microarray platform | Experimental conditions | GEO number, website, or reference |
|---|---|---|---|---|---|
| Injured retina | Retinal tear | Rat | U34A | 4 h; and 1, 3, 7, & 30 d | GSE1001 [1] |
| Injured brain | Cortical impact | Rat | U34A | 3 h | GSE1911 [6,7] |
| Injured spinal cord | Cord contusion | Rat | U34A | 4 h | GSE464 [8] |
| Optic nerve head astrocyte | Cell culture | Human | U95Av2 | Cell culture | GDS532 [17] |
| Cortical astrocyte | Cell culture | Rat | U34A | Cell culture | GSM34300 |
| Brain microglia | Cell culture | Rat | U34A | Cell culture | [18,19] |
| Neuron | Cell culture | Mouse | U74Av2 | Cell culture | JAJohnson [20] |
| Developing retina | Development | Mouse | U74Av2 | P0, P4, P8, P10, P12, P14, P21, & adult | RetDevGE [21] |
| Adult forebrain | Genetic variation | Mouse | U74Av2 | C57BL/6, DBA2/J, F1, & 32 BXD RI strains | [9,12] |

We performed a meta-analysis of previous experimental data to select a group of acute phase genes that are part of a common response to injury in neural tissues. Previously, we examined transcript levels in injured retina, injured brain, cultured astrocytes, and in forebrains from a panel of 35 BXD RI strains [1,6,7,9,12,17]. Microarray data sets describing transcript levels in injured spinal cord, cultured microglia, cultured neurons, and developing retina were obtained from publicly available published studies [8,18–21]. All datasets are available online at Gene Expression Omnibus (GEO).

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**Table 2**

Acute phase genes expressed in the injured CNS

| Symbol | Name (alias) | Astrocytic expression | Microglial expression | Neuronal expression | Chronic expression |
|---|---|---|---|---|---|
| Transcription | | | | | |
| Atf3 | Activating transcription factor 3 | + | + | − | − |
| Btg2 | B-cell translocation gene 2 | + | − | + | − |
| Crem | cAMP responsive element modulator | − | − | − | − |
| Egr1 | Early growth response 1 (Krox-24/NGFI-A) | + | − | + | − |
| Fos | FBJ osteosarcoma oncogene | + | + | + | − |
| Fosl1 | Fos-like 1 | + | − | − | − |
| Junb | Jun-B oncogene | + | − | + | − |
| Ier3 | Immediate early response 3 (PRG1) | + | + | + | − |
| Nr4a1 | Nuclear receptor subfamily 4a1 (NGFI-B/nurr7) | + | − | + | − |
| Cebpd | CCAAT/enhancer binding protein delta | + | − | + | + |
| Id2 | Inhibitor of DNA binding 2 (Idb2) | + | + | + | + |
| Irf1 | Interferon regulatory factor 1 | + | + | − | + |
| Nfkb1 | Nuclear factor kappa B p105 | + | + | − | + |
| Stat3 | Signal transducer and activator of transcription 3 | + | − | + | + |
| Cytokine | | | | | |
| Cxcl10 | Chemokine (C-X-C motif) ligand 10 | + | + | − | + |
| Il1a | Interleukin 1 alpha | − | + | − | − |
| Il1b | Interleukin 1, beta | − | + | − | + |
| Scya2 | Small inducible cytokine A2 (Ccl2) | − | + | − | − |
| Scya3 | Small inducible cytokine A3 (Ccl3) | + | − | − | − |
| Cell adhesion/ECM | | | | | |
| Cd44 | Cd44 antigen | + | + | − | + |
| Icam1 | intercellular adhesion molecule-1 | + | + | − | + |
| Plat | Plasminogen activator tissue | + | + | − | + |
| Serpine1 | Serine proteinase inhibitor-1 (Pai1) | + | − | − | + |
| Timp1 | Tissue inhibitor of metalloproteinase-1 | + | + | − | + |

| Symbol | Name (alias) | Astrocytic expression | Microglial expression | Neuronal expression | Chronic expression |
|---|---|---|---|---|---|
| Metabolism | | | | | |
| Hmox1 | Heme oxygenase | + | + | + | + |
| Mat2a | Methionine adenosyltransferase IIa | + | − | − | − |
| Ptgs2 | Prostaglandin synthase 2 (Cox-2) | + | − | − | − |
| Other | | | | | |
| Ptpn16 | Tyrosine phosphatase nonreceptor type 16 (Dusp1/Ptp) | + | − | + | − |
| Ifrd1 | interferon-related developmental regulator 1 | + | − | + | − |

Acute phase genes displayed transcript changes in injured retina, brain, and spinal cord. Astrocytic, microglial, and neuronal expressions refer to moderate to high transcript levels in cultured astrocytes [17, 20], microglia [18,19], and neurons [20].