

Astrogliosis during acute and chronic cuprizone demyelination and implications for remyelination

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SUPPLEMENTARY DATA

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Abbreviations: BMP4, bone morphogenetic protein 4; CNS, central nervous system; CSPG, chondroitin sulfate proteoglycan; C_t, threshold cycle; Cy3, indocarbocyanine; FGF2, fibroblast growth factor 2; Fn1, fibronectin; GAG, glycosaminoglycan; GFAP, glial fibrillary acidic protein; Glu1, glutamine synthetase; IGF-1, insulin-like growth factor 1; IL-1 β , interleukin 1 β ; Itgam, integrin alpha M; LIF, leukaemia inhibitory factor; LPS, lipopolysaccharide; MCAO, middle cerebral artery occlusion; MOG, myelin oligodendrocyte glycoprotein; Nes, nestin; PFA, paraformaldehyde; Plp1, proteolipid protein; Ptprz1, phosphacan; QPCR, quantitative PCR; TGF β , transforming growth factor β ; TnC, tenascin C; TNF α , tumour necrosis factor α ; Vim, vimentin.

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Table S1 Names, symbols and reference identification numbers for genes included in expression analysis

Gene name	Gene Symbol	Ref Seq ID
Glial fibrillary acidic protein	Gfap	NM_010277
Vimentin	Vim	NM_011701
Nestin	Nes	NM_016701
S100 protein, β -polypeptide, neural	S100b	NM_009115
Glutamine synthetase	Glul	NM_008131
Sox9	Sox9	NM_011448
Laminin, α 1	Lama1	NM_008480
Fibronectin 1	Fn1	NM_010233
Tenascin C	Tnc	NM_011607
Tenascin R	Tnr	NM_022312
Chondroitin sulfate proteoglycan 4 (NG2)	Cspg4	NM_139001
Protein tyrosine phosphatase, receptor type Z, polypeptide 1 (phosphacan)	Ptprz1	NM_001081306
Aggrecan	Acan	NM_007424
Brevican	Bcan	NM_007529
Neurocan	Ncan	NM_007789
Xylosyltransferase-I	Xylt1	NM_175645
Xylosyltransferase-II	Xylt2	NM_145828
Collagen (procollagen, type VIII, α 2)	Col8a1	NM_199473
L1 cell adhesion molecule	L1	NM_008478
Cell adhesion molecule with homology to L1CAM	Chl1	X94310
Integrin α M (Mac 1; CR3)	Itgam	NM_001082960
Endothelin 1	Edn1	NM_010104
Endothelin receptor type A	Ednra	NM_010332
Transforming growth factor, β 1	Tgfb1	NM_011577
Transforming growth factor, β -receptor I	TgfbRI	NM_009370
Transforming growth factor, β -receptor II	TgfbRII	NM_009371
Transforming growth factor, β -receptor III	TgfbRIII	NM_011578
Fibroblast growth factor 2	Fgf2	NM_008006
Fibroblast growth factor receptor 1	Fgfr1	NM_010206
Vascular endothelial growth factor A	Vegfa	NM_001025250
Insulin-like growth factor 1	Igf1	NM_010512
Insulin-like growth factor I receptor	Igf1r	NM_010513
Bone morphogenetic protein 2	Bmp2	NM_007553
Bone morphogenetic protein 4	Bmp4	NM_007554
Bone morphogenetic protein 7	Bmp7	NM_007557
Bone morphogenetic protein receptor, type 1A	Bmpr1a	NM_009758
Bone morphogenetic protein receptor, type 1B	Bmpr1b	NM_007560
Leukaemia inhibitory factor	Lif	NM_008501
Leukaemia inhibitory factor receptor	Lifr	NM_013584
Interleukin 1 β	Il1b	NM_008361
Interleukin 11	Il11	NM_008350
Interleukin 11 receptor, α chain 1	Il11r	NM_010549
Epidermal growth factor	Egf	NM_010113
Epidermal growth factor receptor	Egfr	NM_007912
Tumour necrosis factor	Tnf	NM_013693
Tumour necrosis factor receptor superfamily, member 1a	Tnfr1a	NM_011609
Tumour necrosis factor receptor superfamily, member 1b	Tnfr1b	NM_011610
Ciliary neurotrophic factor	Cntf	NM_170786
Ciliary neurotrophic factor receptor	Cntfr	NM_016673
Brain derived neurotrophic factor	Bdnf	NM_007540
neurotrophin 3	Nt3	NM_008742
Nerve growth factor receptor (p75NTR)	Ngfr	XM_359374
Sonic hedgehog	Shh	NM_009170
growth arrest specific 6	Gas6	NM_019521
TYRO3 protein tyrosine kinase 3	Tyro3	NM_019392
AXL receptor tyrosine kinase	Axl	NM_009465
c-mer proto-oncogene tyrosine kinase	Mer	NM_008587
Lymphotoxin A	Lta	NM_010735
Lymphotoxin B receptor	Ltbr	NM_010736
PPAR δ	Ppard	NM_006238
Notch1	Notch1	NM_008714
Wingless-related MMTV integration site 3A	Wnt3a	NM_009522
Myelin gene regulatory factor (MRF or GM98)	Gm98	XM_359374
Proteolipid protein (myelin) 1	Plp1	NM_011123
Housekeeping genes		
TATA box binding protein	Tbp	NM_013684
Hypoxanthine guanine phosphoribosyl transferase 1	Hprt1	NM_013556
Actin, β , cytoplasmic	Actb	NM_007393
18s RNA	18s Rna	K01364

Table S2 Genes on custom array that exhibited an expression pattern sorted in GenePattern into clusters different than GFAP and the other genes shown in Tables 1 and 2

The order of genes listed is according to the hierarchical clustering to GFAP shown for the chronic time course heat map. The values of 0, 3 and 6 weeks are included in both the acute (top row) and chronic (bottom row) time courses for each gene. The 9- and 12-week time points are not applicable (n.a.) to the acute time course. For the column labelled '20/26 week no cup', the upper rows show values for 20-week-old mice (non-treated mice age-matched to mice that were started on cuprizone at eight weeks of age, then treated with cuprizone for 6 weeks and allowed to recover for 6 weeks). The bottom rows show values for 26-week-old mice (non-treated mice age-matched to mice that were treated with cuprizone for 12 weeks and allowed to recover for six weeks). Values shown are fold change (means \pm S.E.M.) calculated relative to 0 week no cuprizone condition. $n=3$ mice per condition with samples run across three plates as technical triplicates. ANOVA values are for comparison across all time points for a given gene. *Show significant changes ($P<0.05$) for individual time points using comparison with a theoretical mean of 1.000, i.e. null hypothesis for no-fold change. cup, cuprizone.

Gene	0 week no cup	3 week cup	6 week cup	9 week cup	12 week cup	3 week off	6 week off	20/26 week no cup	ANOVA P value
S100b	1.00 \pm 0.065	1.65* \pm 0.054	0.99 \pm 0.052	n.a 0.84 \pm 0.08	n.a 1.14 \pm 0.08	1.13 \pm 0.056 1.56 \pm 0.34	1.80 \pm 0.19 1.25 \pm 0.17	1.25 \pm 0.21 1.57* \pm 0.07	0.002 0.01
Glul	1.00 \pm 0.08	0.96 \pm 0.10	0.91 \pm 0.03	n.a 0.48* \pm 0.01	n.a 0.95 \pm 0.06	0.90 \pm 0.04 1.03 \pm 0.03	1.19 \pm 0.16 0.98 \pm 0.09	1.11 \pm 0.05 0.88 \pm 0.05	0.24 0.0004
Col8a2	1.00 \pm 0.02	1.09 \pm 0.15	0.90 \pm 0.27	n.a 0.81 \pm 0.09	n.a 2.24* \pm 0.12	2.17* \pm 0.22 3.36* \pm 0.22	2.51* \pm 0.08 0.91 \pm 0.06	1.48 \pm 0.25 1.09 \pm 0.10	0.0002 <0.0001
Lama1	1.00 \pm 0.05	1.03 \pm 0.26	1.93* \pm 0.17	n.a 0.70* \pm 0.04	n.a 1.71 \pm 0.27	1.42 \pm 0.16 1.57 \pm 0.19	1.72 \pm 0.18 0.66* \pm 0.05	1.93 \pm 0.23 0.72 \pm 0.13	0.01 0.004
Tnr	1.00 \pm 0.05	1.62 \pm 0.18	0.90 \pm 0.10	n.a 0.60* \pm 0.07	n.a 0.90 \pm 0.08	0.79* \pm 0.03 1.07 \pm 0.07	1.02 \pm 0.16 0.70 \pm 0.10	1.07 \pm 0.09 0.94 \pm 0.06	0.004 0.0004
Acan	1.00 \pm 0.06	1.26 \pm 0.25	1.36* \pm 0.02	n.a 1.03 \pm 0.05	n.a 1.61* \pm 0.10	1.39* \pm 0.06 1.67* \pm 0.11	0.94 \pm 0.22 0.98 \pm 0.07	1.67 \pm 0.24 1.15 \pm 0.22	0.09 0.01
Bcan	1.00 \pm 0.014	1.27* \pm 0.014	0.96 \pm 0.06	n.a 0.74 \pm 0.06	n.a 0.88 \pm 0.06	0.80* \pm 0.009 0.99 \pm 0.17	1.03* \pm 0.01 0.88 \pm 0.04	0.86 \pm 0.09 0.75 \pm 0.07	0.0002 0.01
Ncan	1.00 \pm 0.19	1.01 \pm 0.02	0.72* \pm 0.02	n.a 0.75 \pm 0.09	n.a 0.92 \pm 0.07	0.88 \pm 0.05 0.81 \pm 0.16	1.03 \pm 0.07 0.96 \pm 0.01	1.30 \pm 0.10 0.98 \pm 0.10	0.02 0.33
Cspg4	1.00 \pm 0.05	1.41* \pm 0.08	0.75 \pm 0.08	n.a 0.72* \pm 0.04	n.a 0.67* \pm 0.03	0.75* \pm 0.05 1.23* \pm 0.03	0.96 \pm 0.04 1.10 \pm 0.07	0.83 \pm 0.07 1.02 \pm 0.02	<0.0001 0.002
Xylt1	1.00 \pm 0.12	1.08 \pm 0.13	0.73 \pm 0.09	n.a 0.81* \pm 0.03	n.a 1.27 \pm 0.10	0.81* \pm 0.01 1.21 \pm 0.06	1.08 \pm 0.12 1.08 \pm 0.05	1.36 \pm 0.09 0.91 \pm 0.14	0.01 <0.0001
Xylt2	1.00 \pm 0.13	0.84 \pm 0.06	0.63* \pm 0.03	n.a 0.52* \pm 0.09	n.a 0.77* \pm 0.05	0.71* \pm 0.03 1.42 \pm 0.12	1.17 \pm 0.11 1.25* \pm 0.01	1.02 \pm 0.08 1.11 \pm 0.08	0.005 0.008
Tgfb3	1.00 \pm 0.19	1.24 \pm 0.15	1.69 \pm 0.29	n.a 1.31 \pm 0.18	n.a 1.90* \pm 0.14	1.97 \pm 0.29 2.54* \pm 0.32	1.88 \pm 0.23 1.37 \pm 0.29	1.55* \pm 0.11 1.65 \pm 0.16	0.06 0.004
Bmp2	1.00 \pm 0.18	0.30* \pm 0.02	0.52* \pm 0.03	n.a 0.20* \pm 0.01	n.a 0.54* \pm 0.05	0.54* \pm 0.06 0.78 \pm 0.07	0.90 \pm 0.06 0.57* \pm 0.06	0.90 \pm 0.11 0.89 \pm 0.12	0.002 0.0003
Bmp7	1.00 \pm 0.04	1.18 \pm 0.07	1.82* \pm 0.07	n.a 0.82 \pm 0.05	n.a 1.09 \pm 0.09	1.41 \pm 0.21 1.82* \pm 0.11	1.93* \pm 0.08 1.27 \pm 0.11	1.62* \pm 0.14 0.93 \pm 0.07	0.0005 <0.0001
Bmpr1a	1.00 \pm 0.04	1.33* \pm 0.06	1.00 \pm 0.03	n.a 0.66* \pm 0.03	n.a 1.15 \pm 0.06	1.14 \pm 0.10 1.32* \pm 0.03	1.26* \pm 0.05 1.12 \pm 0.04	1.27* \pm 0.02 0.97 \pm 0.11	0.004 <0.0001
Bmpr1b	1.00 \pm 0.05	1.49 \pm 0.20	0.86 \pm 0.08	n.a 0.65* \pm 0.08	n.a 1.09 \pm 0.04	1.23* \pm 0.01 1.02 \pm 0.12	1.28 \pm 0.17 1.27 \pm 0.08	1.06 \pm 0.04 0.82 \pm 0.13	0.03 0.004
LifR	1.00 \pm 0.01	0.84 \pm 0.05	0.84* \pm 0.01	n.a 0.63* \pm 0.05	n.a 0.99 \pm 0.04	1.00 \pm 0.02 1.05 \pm 0.07	1.01 \pm 0.02 1.02 \pm 0.07	1.33* \pm 0.06 1.16 \pm 0.05	<0.0001 0.0004
Cntf	1.00 \pm 0.08	1.06 \pm 0.07	0.71* \pm 0.04	n.a 0.58* \pm 0.02	n.a 0.74* \pm 0.015	0.67* \pm 0.03 0.83 \pm 0.091	1.10 \pm 0.09 1.02 \pm 0.12	1.05 \pm 0.05 0.91 \pm 0.094	0.0009 0.001
Cntfr	1.00 \pm 0.03	0.91 \pm 0.07	0.91* \pm 0.01	n.a 0.83 \pm 0.06	n.a 1.06 \pm 0.13	1.02 \pm 0.08 1.39* \pm 0.02	1.24 \pm 0.01 1.02 \pm 0.02	1.25* \pm 0.01 1.08 \pm 0.09	0.004 0.0008
Vegfa	1.00 \pm 0.03	0.68* \pm 0.02	0.70 \pm 0.08	n.a 0.50* \pm 0.06	n.a 0.73* \pm 0.03	0.61* \pm 0.01 0.74 \pm 0.09	0.80* \pm 0.05 1.03 \pm 0.07	0.88 \pm 0.04 1.12 \pm 0.08	0.0005 0.002
Fgfr1	1 \pm 0.11	1.74 \pm 0.33	1.88* \pm 0.18	n.a 1.33 \pm 0.09	n.a 1.91* \pm 0.12	1.89* \pm 0.06 1.92 \pm 0.30	1.82* \pm 0.18 1.38 \pm 0.13	1.51* \pm 0.04 1.28* \pm 0.06	0.03 0.043
NgfR	1.00 \pm 0.03	1.42 \pm 0.11	1.96* \pm 0.19	n.a 1.72* \pm 0.16	n.a 1.37 \pm 0.18	1.12 \pm 0.10 1.60* \pm 0.09	1.35 \pm 0.30 1.44 \pm 0.21	1.98 \pm 0.23 1.69* \pm 0.11	0.01 0.006
Ngf	1.00 \pm 0.05	1.16 \pm 0.05	0.80 \pm 0.14	n.a 0.82 \pm 0.07	n.a 1.08 \pm 0.03	1.04 \pm 0.13 1.77* \pm 0.15	1.31* \pm 0.06 1.27 \pm 0.10	1.26 \pm 0.14 0.98 \pm 0.05	0.04 0.0001

Table S2 Continued.

Gene	0 week no cup	3 week cup	6 week cup	9 week cup	12 week cup	3 week off	6 week off	20/26 week no cup	ANOVA P value
Bdnf	1.00 ± 0.06	1.56* ± 0.11	1.53* ± 0.10	n.a 0.93 ± 0.04	n.a 2.10* ± 0.18	1.69 ± 0.43 1.66* ± 0.02	1.70 ± 0.23 0.80 ± 0.09	2.75* ± 0.19 0.84 ± 0.06	0.004 <0.0001
Ntf3	1.00 ± 0.07	5.59* ± 0.27	8.73* ± 0.33	n.a 4.65* ± 0.25	n.a 7.37* ± 0.62	10.88* ± 1.37 11.91* ± 0.62	3.92* ± 0.50 1.25* ± 0.04	2.09* ± 0.24 1.85* ± 0.13	<0.0001 <0.0001
Igf1r	1.00 ± 0.04	0.78* ± 0.04	0.67* ± 0.03	n.a 0.55* ± .04	n.a 0.96 ± 0.11	0.75 ± 0.09 0.99 ± 0.10	1.06 ± 0.12 0.96 ± 0.09	1.11 ± 0.04 1.29 ± 0.22	0.002 0.002
Egf	1.00 ± 0.17	0.76 ± 0.16	0.58 ± 0.12	n.a 0.46* ± 0.09	n.a 0.89 ± 0.13	0.57* ± 0.07 1.10 ± 0.09	1.10 ± 0.11 1.61 ± 0.30	1.08 ± 0.12 1.14 ± 0.07	0.04 0.03
Egfr	1.00 ± 0.01	1.23* ± 0.04	1.10 ± 0.16	n.a 0.88 ± 0.05	n.a 1.49* ± 0.05	0.78 ± 0.19 1.76 ± 0.18	1.22 ± 0.30 1.22* ± 0.01	1.27 ± 0.17 1.19* ± 0.03	0.41 0.0006
Edn1	1.00 ± 0.02	0.26* ± 0.03	0.21* ± 0.06	n.a 0.17* ± 0.01	n.a 0.29* ± 0.04	0.36* ± 0.02 0.61* ± 0.09	0.63* ± 0.06 0.85 ± 0.16	0.77 ± 0.07 1.04 ± 0.14	<0.0001 <0.0001
Ednra	1.00 ± 0.06	1.91 ± 0.24	1.16 ± 0.17	n.a 1.27 ± 0.13	n.a 1.03 ± 0.03	1.62 ± 0.16 2.14* ± 0.06	1.51 ± 0.15 1.47 ± 0.20	1.59* ± 0.11 1.12 ± 0.05	0.02 0.02
Tnfsf13	1.00 ± 0.03	1.32 ± 0.22	1.76* ± 0.16	n.a 0.68 ± 0.12	n.a 1.51* ± 0.03	1.07 ± 0.11 1.53 ± 0.19	1.74 ± 0.22 0.96 ± 0.11	1.22* ± 0.05 0.87 ± 0.05	0.01 0.0002
Lta	1.00 ± 0.17	0.72* ± 0.06	0.69 ± 0.14	n.a 0.50* ± 0.08	n.a 0.93 ± 0.29	0.64 ± 0.25 1.05 ± 0.09	1.36 ± 0.18 1.26 ± 0.13	1.11 ± 0.40 0.95 ± 0.57	0.25 0.0019
Il11	1.00 ± 0.14	0.26* ± 0.06	0.37* ± 0.04	n.a 0.31* ± 0.06	n.a 0.36* ± 0.12	0.43* ± 0.13 0.79 ± 0.08	1.01 ± 0.16 0.84 ± 0.14	0.99 ± 0.12 0.81 ± 0.21	0.0007 0.20
Il11ra1	1.00 ± 0.07	1.32 ± 0.10	0.98 ± 0.19	n.a 0.67 ± 0.10	n.a 0.97 ± 0.21	0.70 ± 0.07 1.39* ± 0.08	1.19 ± 0.05 0.93 ± 0.07	0.99 ± 0.02 0.88* ± 0.01	0.01 0.0004
Shh	1.00 ± 0.06	0.48* ± 0.03	0.79 ± 0.06	n.a 0.71 ± 0.10	n.a 0.76* ± 0.03	0.67* ± 0.08 1.03 ± 0.11	1.33 ± 0.12 1.00 ± 0.018	1.47 ± 0.17 1.24 ± 0.13	<0.0001 0.03
L1cam	1.00 ± 0.08	0.65* ± 0.04	0.72* ± 0.03	n.a 0.59* ± 0.08	n.a 0.94 ± 0.11	0.71 ± 0.20 1.18 ± 0.08	1.15 ± 0.24 1.06 ± 0.09	1.31 ± 0.09 1.00 ± 0.02	0.03 0.0002
Chl1	1.00 ± 0.04	1.18 ± 0.08	1.32* ± 0.07	n.a 0.79 ± 0.06	n.a 1.36* ± 0.06	1.20* ± 0.04 1.26 ± 0.21	1.15 ± 0.18 1.05 ± 0.04	1.33* ± 0.03 0.83 ± 0.04	0.19 0.0009
Notch1	1.00 ± 0.11	0.91 ± 0.07	0.89 ± 0.11	n.a 1.24 ± 0.10	n.a 1.25 ± 0.25	0.94 ± 0.27 1.18* ± 0.04	0.84 ± 0.09 1.26 ± 0.27	0.78 ± 0.08 0.86 ± 0.03	0.90 0.02
Ppard	1.00 ± 0.02	1.03 ± 0.08	0.86 ± 0.06	n.a 0.74* ± 0.02	n.a 0.95 ± 0.03	0.77* ± 0.01 0.93 ± 0.10	0.89 ± 0.15 0.78 ± 0.08	1.29 ± 0.07 1.07 ± 0.05	0.01 0.23
Wnt3a	1.00 ± 0.34	0.94 ± 0.22	0.52 ± 0.19	n.a 0.83 ± 0.17	n.a 0.50* ± 0.09	0.74 ± 0.18 1.68* ± 0.16	0.86 ± 0.23 1.32 ± 0.33	0.57 ± 0.25 0.58* ± 0.09	0.66 0.05
Gas6	1.00 ± 0.12	0.95 ± 0.18	0.94* ± 0.01	n.a 0.68* ± 0.06	n.a 0.99 ± 0.01	0.81 ± 0.07 1.07 ± 0.22	0.97 ± 0.25 0.86 ± 0.04	1.28 ± 0.20 0.92 ± 0.04	0.49 0.02
Tyro3	1.00 ± 0.05	0.61 ± 0.13	0.54* ± 0.01	n.a 0.39* ± 0.03	n.a 0.64* ± 0.02	0.47* ± 0.09 1.10 ± 0.23	0.99 ± 0.19 0.67* ± 0.06	1.16 ± 0.11 0.79 ± 0.09	0.005 0.40
Gm98	1.00 ± 0.06	0.74 ± 0.10	0.43* ± 0.05	n.a 0.40* ± 0.03	n.a 0.22* ± 0.01	0.37* ± 0.04 0.66* ± 0.05	0.82 ± 0.11 1.30 ± 0.21	0.96 ± 0.08 1.74 ± 0.28	0.0003 0.004
Plp1	1.00 ± 0.05	0.71* ± 0.03	0.18* ± 0.01	n.a 0.14* ± 0.01	n.a 0.11* ± 0.01	0.67* ± 0.02 0.69* ± 0.01	0.78* ± 0.02 0.89 ± 0.05	0.73* ± 0.01 0.89 ± 0.07	<0.0001 <0.0001