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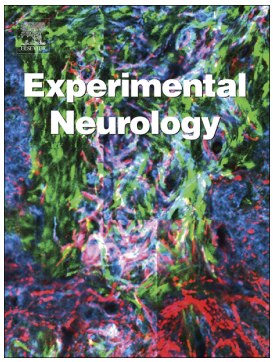

Molecular pathology of Multiple Sclerosis lesions reveals a heterogeneous expression pattern of genes involved in oligodendrogliogenesis

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Article

Title: Molecular pathology of Multiple Sclerosis lesions reveals a heterogeneous expression pattern of genes involved in oligodendrogliogenesis.

Abbreviated title: Gene expression analysis of MS WM lesions

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Abbreviations: AL, active lesion; RL, remyelinating lesion; IdL, Inactive demyelinating lesion; NAWM, normal appearing white matter

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Abstract

Little is known about the decisive molecular factors that regulate lesion remyelination in Multiple Sclerosis. To identify such factors, we performed a differential gene expression analysis of normal appearing white matter (NAWM), active, remyelinating, and inactive demyelinated lesions. As expected, many genes involved in inflammatory processes were detected to be differentially regulated between these tissue types. Among them, we found an increased expression of members of the STAT6 pathway such as STAT6, IL4 and IL4R in active, remyelinated and inactive demyelinated lesions. This suggests that a protective, anti-inflammatory reaction, as already reported to be present in MS NAWM, is further enhanced in lesion tissues. Focusing on genes influencing oligodendrogliogenesis, we found a decreased expression of NKX2-2 in active, remyelinated and inactive demyelinated lesions, whereas SOX10 was downregulated in inactive demyelinated lesions, when compared to NAWM. Simultaneously, CXCL12 (SDF1) expression was strongly increased in active, remyelinated and inactive demyelinated lesions, but increased expression of the IGF1 and IGF2 genes was found in inactive demyelinated lesions. This demonstrates that, in principle, expression of genes promoting oligodendrogliogenesis occurs in MS lesion tissue - even in inactive demyelinated lesions. In contrast, oligodendrogenesis inhibiting genes such as JAG1 were also expressed at higher levels in inactive demyelinated lesions. Both, oligodendrogliogenesis promoting as well as inhibiting genes are expressed in all lesion tissues. However, no clear promoting or inhibiting expression pattern could be detected in any of the different types of lesioned tissues. This might reflect the heterogeneity of lesion development in MS patients, both in terms of mechanisms and temporal differences.

Keywords: Multiple Sclerosis, Gene expression analysis, Molecular pathology, Oligodendrogliogenesis, STAT6 pathway, Multiple Sclerosis lesions

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Introduction

Multiple Sclerosis (MS) is a chronic inflammatory, demyelinating disease of the central nervous system (CNS) with a large degree of heterogeneity in clinical course and histopathology (Compston and Coles, 2002). It is suggested that MS is an autoimmune disorder directed against CNS antigens, which leads to inflammation and demyelination (Hemmer et al., 2002; Noseworthy et al., 2000). However, other findings suggest that the primary cause for inflammation in MS could be primary oligodendrocyte degeneration (Barnett and Prineas, 2004; Trapp, 2004). One major characteristic of MS is the formation of the so-called plaques or lesions; areas of demyelination in the white matter with variable levels of axonal damage (Lassmann, 1998) (Figure1). The mechanisms underlying early lesion development are still unclear, but are thought to involve altered BBB permeability and nodules of activated microglial (van der Valk and Amor, 2009) in the normal appearing tissue, in which sub-pathological changes have been shown to be present (Filippi et al., 1998; Graumann et al., 2003; Lindberg et al., 2004; Zeis et al., 2008). Some of these areas of microglial activation are suggested to transform into full, active demyelinating lesions characterized by massive infiltration of immune cells, primarily of the monocyte/macrophage lineage.

There is evidence that remyelination in the MS brain can be extensive, although highly heterogeneous (Trapp, 2004), and may be initiated in rapid succession following demyelination (Prineas et al., 1989; Raine and Wu, 1993). Depending on whether the process of repair and remyelination prevails over demyelination or not, active lesions develop into inactive demyelinated lesions, partially remyelinated or fully remyelinated lesions. To remyelinate, oligodendrocyte progenitor cells (OPCs) are recruited to repopulate the area of demyelination, differentiate into oligodendrocytes and remyelinate the demyelinated axons (Franklin, 2002). During these steps, OPCs have to migrate, proliferate and differentiate; processes that have to be tightly controlled by numerous molecular events, including axonal electrical activity (Franklin, 2002; Franklin and Ffrench-Constant, 2008; Gautier et al., 2015). Failure of remyelination results in inactive demyelinated lesions, in which myelin has been removed, immune cells are largely absent and the remaining demyelinated axons are embedded in a glial scar (Compston et al., 2006).

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Substantial progress has been made in understanding the molecular mechanisms of remyelination. However, the overall nature and timing of molecular signaling influencing remyelination in MS lesion development and resolution are still incompletely understood. To identify genes involved in lesion formation and resolution, we performed a differential gene expression analysis between the distinct histological tissue types from brain autopsies of MS cases: normal appearing white matter (NAWM), active lesions (AL), remyelinating lesions (RL) and inactive demyelinated lesions (IdL). We found several genes involved in inflammatory processes to be differentially regulated. We further aimed to identify genes known to be involved in oligodendrogliogenesis in order to identify potential key factors in oligodendrocyte repair/remyelination. In all tissues, we found altered expression of genes both promoting and inhibiting oligodendrogliogenesis. Our study shows that the molecular pattern of pathohistologically similar tissues can be quite variable, possibly reflecting the heterogeneity of lesion development and repair in MS brain tissue.

Materials and Methods

Tissue collection

MS and control tissue samples were supplied by the UK Multiple Sclerosis Tissue Bank (UK Multicentre Research Ethics Committee, MREC/02/2/39), funded by the Multiple Sclerosis Society of Great Britain and Northern Ireland (registered charity 207495). For this study, tissue samples representing different tissue types were isolated from a total of 7 progressive MS cases (4 SPMS, 3 PPMS; Table 1). The median disease duration was 21 years (range from 11 to 27). The median age at death was 40 years (range from 34 to 49). Altogether, 7 remyelinating lesions, 9 NAWM regions, 9 active lesions and 5 inactive demyelinated lesions were dissected (see Table 1). All brains had been screened by a neuropathologist to confirm diagnosis of MS and to exclude other confounding pathologies. All tissues were screened for the presence of T-cells (CD3), activated microglia and macrophages (CD68), activated astrocytes (GFAP) and signs of demyelination and remyelination (MOG, PLP) (Figure 1). Regions of interest for RNA isolation were dissected manually from several 20μm cryosections per block. In regular intervals, sections

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were analysed by immunohistochemistry to confirm tissue characteristics and to exclude contamination of the dissected tissue sample with other tissue types.

Tissue characterization and definition

Immunostaining against MOG and HLA-DR, as well as Luxol fast blue (LFB) histology, were performed to characterize and differentiate the tissue types (Figure 1), as suggested by Kuhlmann et al (Kuhlmann et al., 2017). NAWM tissue was characterized by a normal myelin (Figure 1A) and microglial staining at least 10mm from areas of visible demyelination (Figure 1A, B). Active lesions (AL) showed a loss of myelin (Figure 1C) and the presence of activated microglia/macrophages containing myelin debris (Figure 1D, E). Remyelinating tissue was characterized by pale or thinly myelinated fibres with MOG+ oligodendrocytes in areas of ongoing inflammation (Figure 1F, G and H) or characterized by areas of pale LFB+ myelin staining (Figure 1I, J) with some residual activated microglia/macrophages (Figure 1K). Inactive demyelinated lesions (IdL) were completely demyelinated with a well demarcated border (Figure 1L) and very few macrophages (Figure 1M).

Immunohistochemistry

All tissue samples were analysed by immunohistochemistry. Cryostat sections (10μm) used for tissue characterization using anti-HLA-DR, -MBP and -MOG antibodies were fixed for 10min in 10% formalin. For inactivation of endogenous peroxidase, all sections were treated with 0.3% hydrogen peroxide and blocked with blocking buffer (1% normal donkey serum, 2% Fish skin gelatin, 0.15% Triton). After quenching, sections were incubated with primary antibodies overnight at 4°C. Secondary biotinylated antibodies were applied for 1 hour at room temperature followed by the ABC complex reagent (Vector Labs) for 1 hour. Color reaction was performed with 3-Amino-9-ethylcarbazole or diaminobenzidine (Erne et al., 2002). Counterstaining was performed in haematoxylin for 1 minute followed by rinsing the slide in running tap water. Antibodies used are shown in Table 2.

Total RNA preparation

Serial sections (20μm) were prepared, following tissue characterization and identification of regions of interest (above), for the isolation of total RNA. Tissue was isolated by micro-dissecting areas of NAWM, AL, aRL, cRL or Idl with reference to

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the characterized sections from a minimum of 3 serial 20μm sections. At regular intervals, sections were analysed by immunohistochemistry to confirm tissue characteristics and to exclude contamination of tissue sample with other tissue types. Samples were handled and cut in such a way that they never thawed and the cryostat, dissecting equipment and collection tubes were treated with RNaseZap (ThermoFisher Scientific) or certified free of RNase/ DNase contamination. Total RNA isolation was performed by homogenizing the tissue in guanidinium thiocyanate followed by a CsCl ultracentrifugation (Graumann et al., 2003). Freshly isolated RNA was tested for integrity using an Agilent 2100 Bioanalyser instrument (Agilent, Santa Clara, California, USA; all samples had an RNA Integrity Number of >7) and by Northern blot analysis for the expression of GFAP mRNA, as previously described (Graumann et al., 2003).

Atlas™ cDNA Expression Array hybridization

For this analysis, a custom made Clontech Atlas™ cDNA Expression Array was made which contains 1'185 selected cDNA sequences on a nylon membrane. Genes spotted on the nylon membrane were based on the data of earlier gene expression studies (Graumann et al., 2003 and Zeis et al., 2008) as well as on literature searches for genes involved in oligodendrogenesis (detailed list is found in Supporting information Table 1) resulting in an array focused on CNS and especially oligodendrogliogenesis relevant genes. Array hybridization was performed according to Graumann et al., (2003). Hybridization signals were detected with the phosphoimaging system (GE Healthcare Europe, Amersham, Molecular Dynamics, Glattbrugg, Switzerland). Differential gene expression analysis was made with 30 total RNA samples of different origin from 7 patients (Table 1). From 1'185 genes present on the array, 1,099 genes were detectable at least in one tissue group (NAWM, AL, RL and IdL), whereas 86 were not detectable in any tissue groups.

qRT-PCR

Quantitative RT-PCR was performed in 384-well plates (VWR, Radnor, USA, Cat.No. 82006-678) using the ABI ViiA Fast Real-Time PCR system (Applied Biosystems, Life technologies Ltd., Paisley, UK). Primer sequences were designed from unique sites over exon–intron junctions to prevent amplification of genomic DNA. Real-time RT-PCR was performed according to the manufacturer's protocol using the KAPA

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SYBR Fast Universal 2x qPCR Master mix (KAPA Biosystems, Woburn, USA). RNA amounts were calculated with relative standard curves for all mRNAs of interest. qRT-PCR was normalized with the H1FX, RPL3 and RPS6KA3 housekeeping genes.

Gene expression analysis, statistics and pathway analysis

Quantification of differential hybridization signal intensities was achieved with the AtlasImage™ 2.0 software program. A log2 transformation was then applied to the raw intensity values of each array. Normalization was completed using the quantile normalization method. Expression data were analysed using R and the software package limma (Phipson et al., 2016; R developmental Core Team, 2008; Ritchie et al., 2015). Statistical analysis was performed using the linear model with tissue type, patient and batch as factors. Genes with a p-value below 0.05 ($p<0.05$) were considered significantly changed between groups. Fold changes above 1.5 are considered to be higher expressed, whereas fold changes below -1.5 are considered to be lower expressed in the different tissue types. Data were further analysed with IPA (Ingenuity® Systems, www.ingenuity.com). All genes with a fold change higher/lower than +/-1.5 were used for the ingenuity pathway analysis. Analysis was performed comparing all different tissue types.

Results

Similarities and differences in gene expression between the different MS tissue types

As a first step, we performed LFB staining as well as a detailed immunohistochemical analysis of all MS brain tissues to identify the different tissue types (NAWM, AL, RL and IdL; Figure 1), a prerequisite for our differential gene expression analysis. All gene expression data were compared between the different tissue types (Figure 2, Supporting information Table 1), representative for specific stages in lesion development (Figure 1).

To identify genes which might be involved in the initiation further development of lesions, we first compared the gene expression patterns between AL and NAWM. From the 1,185 genes present on the array, 47 were significantly differentially expressed ($p < 0.05$, $|FC| > 1.5$) between AL and NAWM (Fig. 2A). Among them, 17 had increased and 30 decreased expression in AL tissue.

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A comparison between RL tissue, which is in the process or at the end of remyelination, and NAWM would be expected to reveal genes involved in remyelination or genes involved in the maintenance of oligodendrocytes. This comparison between RL and NAWM revealed 57 genes to be significantly differentially expressed. Expression of 28 genes was increased, whereas the expression of 29 genes was reduced in RL tissue (Fig. 2B).

Comparing gene expression of NAWM with IdL would be expected to reveal significant reduction in expression of oligodendrocyte specific genes due to the oligodendrocyte loss in IdL. Furthermore, this comparison serves as a positive experimental control, as myelin gene expression was reduced as anticipated in IdL lesions compared to NAWM (Fig. 2C). Finally, genes involved in the initiation or regulation of oligodendrogliogenesis might either be up- or downregulated in IdL. Reflecting the cellular changes, the most significantly differentially expressed genes were found between IdL and NAWM, where 50 genes were increased and 110 genes were decreased in IdL (Fig. 2C).

The comparison of AL and RL potentially reveals genes that are necessary for the initiation of remyelination. However, the fewest significantly differentially expressed genes were found between AL and RL. Only 3 genes were significantly increased and 6 genes significantly decreased in AL tissue in comparison to RL (Fig. 2D). Differences in gene expression between AL and IdL might be expected to reveal genes inhibiting remyelination and/or promoting astrogliogenesis/scar formation. Comparison between AL and IdL revealed 74 significantly differentially expressed genes, of which 55 were increased and 19 decreased, in AL tissue (Fig. 2E). Comparing RL and IdL gene expression should reveal genes involved in remyelination or genes inhibiting remyelination. 33 genes were found to be significantly differentially expressed between RL and IdL tissues. Expression of 23 genes was increased and 10 decreased in RL tissue (Fig. 2F).

A summary of the top 30 most changed genes between all tissue types and possibly the most relevant in lesion development and resolution is shown in Table 3. A

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detailed list of all differentially expressed genes can be found in Supporting information Table 1.

Comparison of remyelinating and chronic silent lesions did not reveal a clear promotion of oligodendrogliogenesis.

In RL tissue, remyelination has either already taken place or is ongoing. In contrast, in IdL it is assumed that remyelination has failed. Therefore, it might be expected that this comparison might reveal genes important for remyelination or inhibition thereof. Analysis of differential gene expression of RL vs IdL, however, did not reveal a clear result. As expected, we detected a reduced expression of several genes known to be expressed in oligodendrocytes in IdL, such as e.g. GJB1, MAG, SOX10 or MAL (Table 4). We then searched for genes influencing oligodendrogliogenesis in RL and found JAG2 expression, known to inhibit oligodendrogliogenesis, to be reduced in RL vs IdL (p=0.02, Table 4). We also found CNTN2 (p=0.01) and SOX10 (0.01), both known to promote oligodendrogliogenesis (Zoupi et al., 2018), to be more highly expressed in RL. In contrast, expression of IGF2 (p=0.01) and MARCKS (p=0.05), both thought to positively influence oligodendrogliogenesis, were reduced in RL than in IdL (Table 4). Altogether, no clear pattern of genes promoting or inhibiting oligodendrogliogenesis was detected between RL and IdL tissue.

Cluster analysis reveals heterogeneity between the different tissue types

To analyze and characterize the differential gene expression between NAWM, AL, RL and IdL in more detail, a cluster analysis was performed (Figure 3). Basically, three clusters were detected. Cluster I mainly included NAWM (Figure 3). Six out of 9 NAWM samples were found in cluster I whereas one was found in cluster II and two in cluster III. Cluster II included most IdL tissue samples of which 4 of 5 were found in this cluster (Figure 3). One IdL sample was found in cluster III. Both AL and RL tissue samples were dispersed throughout all three clusters and did not separate in two separate clusters, despite some similarities in immunohistological appearance. No clustering was detected when the confounding factors, batch and patient, were analysed.

Genes involved in oligodendrogliogenesis are differentially expressed between NAWM, AL, RL and IdL

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For successful remyelination to occur, OPCs have to become activated, recruited to the lesion area and differentiate into remyelinating oligodendrocytes (Franklin and Ffrench-Constant, 2008). By analyzing the differential gene expression pattern, we found altered expression of many genes influencing oligodendrogliogenesis (Table 4, Figure 5) in a positive and negative way in all the different tissue types.

We found the expression of IGF1, reported to influence oligodendrogliogenesis (Beck et al., 1995; Carson et al., 1993; Huang and Dreyfus, 2016; McMorris et al., 1986), to be significantly elevated in IdL in comparison to NAWM ($p=0.019$, Figure 4A). In comparison to NAWM, IGF1 expression was increased in some AL and RL, but this was not a consistent result. IGF1 and IGF2 expression were significantly increased in IdL in comparison to NAWM ($p=0.015$). IGF2 expression was significantly reduced in RL in comparison to IdL ($p=0.008$, Figure 4B). Fibroblast growth factors, such as FGF2 and FGF9, have been reported to be involved in oligodendrocyte maturation (Fortin et al., 2005; Furusho et al., 2012; Lindner et al., 2015; McKinnon et al., 1990). However, we found no significant difference in FGF expression between the analysed tissue types. Some cases showed a strong increase in FGF2 and FGF9, whereas in others FGFs were down regulated. FGFR4 expression showed a trend to be increased in RL vs. NAWM ($p=0.076$, Figure 4C), and FGFR1-3 expression did not significantly change between NAWM, AL, RL and IdL.

Oligodendrocyte differentiation has been shown to be influenced by the notch signaling pathway (Popko, 2003), which can either inhibit (Genoud et al., 2002; Wang et al., 1998) or promote oligodendrocyte maturation (Aparicio et al., 2013; Grandbarbe et al., 2003; Hu et al., 2003), depending on the stimulus. Here, we found a trend towards a higher expression of JAG1 in IdL in comparison to NAWM ($p=0.076$). In contrast, activation by F3/contactin (CNTN1) promotes OPC differentiation / maturation (Aparicio et al., 2013). However, no significant difference in CNTN1 or CNTN2 expression was detected in MS tissues. NOTCH1 expression itself showed a trend towards being reduced in RL ($p=0.06$) in comparison to NAWM.

CXCL12/CXCR4 signaling has been demonstrated to be involved in oligodendrogliogenesis (Dziembowska et al., 2005; Zilkha-Falb et al., 2016). In comparison to NAWM, CXCL12 expression was significantly higher in AL ($p=0.019$),

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RL (p=0.006) as well as IdL (p=0.002) (Figure 4C). In contrast, CXCR4 expression was not significantly changed between the different tissue types. Oligodendrocyte differentiation and maturation is further regulated by NKX2-2 (Qi et al., 2001). In the different tissue types, NKX2-2 expression was significantly lower in AL (p=0.008) and IdL (p=0.003), but not in RL (Figure 4D). The terminal differentiation of oligodendrocytes is dependent on the transcription factor Sox10 (Stolt et al., 2002). The expression of SOX10 was significantly reduced in IdL in comparison to AL (p=0.032), RL (p=0.007) and NAWM (p=0.005, Figure 4E). Finally, SEMA3B has been shown to inhibit oligodendrocyte migration (Cohen et al., 2003). In our gene expression analysis, SEMA3B expression was significantly increased in NAWM in comparison to AL (p=0.019), RL (p=0.041) and IdL (p=0.006, Figure 4F).

Genes involved in the STAT6 signaling pathway are differentially expressed in lesion tissue

We have previously reported that members of the STAT6 signaling pathway are upregulated in NAWM in comparison to control WM (Zeis et al., 2008). Here we show that the expression of members of the STAT6 signaling pathway, such as STAT6 itself, IL4 and IL4R, is increased in AL, RL and IdL tissue (Table 5, Figure 4G, H, I). STAT6 expression is significantly higher in AL, RL and IdL lesions (Figure 4G). IL4 expression showed a strong tendency to be increased in RL in comparison to NAWM (p=0.051) and in AL and IdL (Figure 4H). Finally, IL4R expression was significantly higher in RL and IdL in comparison to NAWM. In AL, IL4R expression showed a trend towards being increased but not in all cases (Figure 4I). Expression of JAK1 and JAK3 did not show any significant expression changes between NAWM, AL, RL and IdL.

Expression of myelin genes decreases from NAWM to IdL tissue

The expression of myelin genes MAG, MAL (Figure 4J), MOBP, MOG (Figure 4K), and PLP1 (Figure 4L), with the exception of MBP, was not significantly changed in AL or RL in comparison to NAWM. In contrast, myelin gene expression was strongly reduced in IdL in comparison to NAWM (Table 4). However, MBP gene expression was significantly increased in RL in comparison to NAWM (Table 4).

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Pathway analysis shows a strong immune activation in all tissues in comparison to NAWM

To investigate possible pathways and/or networks involved in lesion formation or resolution, we performed an Ingenuity pathway core analysis between all different tissue types (Table 6). Compared to NAWM, an increased *proliferation of immune cells* was predicted in all lesion types (activation z-score; AL: 1.806, RL: 2.296, IdL: 2.434). Moreover, an increased activation of many different immune system functions was predicted in AL, RL and IdL in comparison to NAWM (Table 6). Interestingly, a significant activation of *development of neurons* pathways was predicted in AL tissue in comparison to NAWM (p= 2.08E-12, activation z-score=2.672). In addition, *cell viability of neuroglia* was also predicted to be increased in AL (p=3.89E-13, activation z-score=2.260). In the comparison of RL vs NAWM, an increase of *myelination* was predicted (p=3.28E-15, activation z-score=2.170).

Comparison between AL and RL revealed a predicted decrease in several functions associated with immune system function such as, *cell movement of phagocytes* (p=6.26E-27, activation z-score=-3.419) and *leukocyte migration* (p=1.07E-31, activation z-score=-2.768) in the AL tissue. Finally, analysis of the differential gene expression between RL and IdL revealed a predicted increase of *lymphocyte stimulation* (p=1.18E-09, activation z-score=2.211) and genes involved in *systemic autoimmune disease* (p=7.03E-19, activation z-score=2.672).

qRT-PCR verification

To verify the results of the differential gene expression study, we performed a qRT-PCR analysis on a different set of tissues from 9 different MS patients. In total the expression of IGF2, CXCL12, STAT6, NPY, MAG as well as MAL was analysed on 22 NAWM, 14 AL, 8 RL and 9 IdL tissue samples (Table 7). Overall, qRT-PCR confirmed the results from the ATLAS cDNA gene expression array. In the comparison of RL vs NAWM, NPY, STAT6 and MAG showed a similar increase or decrease in expression in qRT-PCR as in the array study, but without significance (NPY; p=0.105, STAT6; p=0.300, MAG; p=0.149).

Discussion

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We have previously shown that there is a balance between proinflammatory genes, expressed mainly by microglia, and anti-inflammatory and neuroprotective genes, expressed predominantly by oligodendrocytes in the MS NAWM in comparison to control (Graumann et al., 2003; Zeis et al., 2008; Zeis et al., 2009). Therefore, it might be expected that a shift in the balance of these opposing processes might lead to the formation of lesions. To investigate this further, we analysed the gene expression of normal appearing white matter (NAWM), active, remyelinating and inactive demyelinating lesions with a focus on genes known to be involved in oligodendrocyte development and regeneration. By comparing the gene expression of the different tissue types, representative for lesion developmental stages, we speculated that depending on the comparison made, genes involved in various processes influencing lesion growth, repair or inactivation might be detected. We expected that genes involved in lesion growth and/or inflammatory processes might be detected in NAWM vs AL, whereas genes playing a role in lesion clearance/repair would be detected in the AL vs RL comparison. Comparing RL vs IdL might have been expected to reveal genes that play a role in either blocking or initiating/promoting remyelination. Our results show that our hypothesis was partially validated (Table 6). However, heterogeneity of gene expression in individual categories and, in retrospect the use of too broad a lesion staging process, made it challenging to detect clear differences between the data sets.

The expression of many genes varied strongly across the different types of white matter tissue seen in the MS brain (NAWM, AL, RL, IdL). For example, the expression of the IGF1 gene varied by more than 128 fold between the different IdL tissue samples (Figure 4A), even though these tissue samples were similar at the immunohistochemical level. This heterogeneity could result from the different stages of lesion development and resolution in the samples, which would lead to changes in cell populations, or different pathogenetic mechanisms. Such variability in the expression of genes influencing oligodendrocyte development might be one of the causes for heterogeneous lesion outcome in MS patients. Lesion specific signaling, originating from the interplay between the different cells present, might lead to the reported phenomenon that in a single patient both successful remyelination and remyelination failure is detected (Patrikios et al., 2006).

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There are potentially many causes for remyelination failure; non-disease-related factors (age, sex, genetic background; (Franklin and Ffrench-Constant, 2008), insufficient OPC availability (Ludwin, 1980; Mason et al., 2004), insufficient OPC migration (Boyd et al., 2013), deficient OPC differentiation, proliferation or myelination (Franklin and Ffrench-Constant, 2008; Wolswijk, 1998) or compromised axons unable to support their remyelination (Chang et al., 2002). Our results show that even in inactive demyelinated lesions, factors are present that could initiate and/or support remyelination (e.g. IGF1, IGF2 or CXCL12). We speculate that, although such factors are present, the simultaneous presence of factors opposing oligodendrocyte development (e.g. JAG1 (Wang et al., 1998)) might shift the balance in favor of demyelination. We also found an elevated expression of genes known to inhibit oligodendrogliogenesis in remyelinating lesion tissue (e.g. EDN1, TNC). We suggest that in this case, the expression of factors promoting remyelination exerted a stronger effect and neutralized the effects of genes inhibiting oligodendrocyte development, leading to successful remyelination. Furthermore, the timing of expression of developmental factors is crucial in oligodendrocyte development (Franklin, 2002; Franklin and Ffrench-Constant, 2008). Many factors can promote specific steps in oligodendrocyte development but have inhibiting properties at other stages. This is true for FGF2 or PDGF, which both promote OPC proliferation. Later however, PDGF slows down and FGF2 even inhibits differentiation (McKinnon et al., 1993). Therefore, genes normally beneficial in oligodendrocyte development might be detrimental for remyelination if expressed at the wrong time point. Altogether, we did not detect a clear pro- or anti- oligodendrogliogenesis/remyelinating gene expression pattern in the different types of lesions (Figure 5).

We previously reported an increased expression of STAT6 in the NAWM of MS patients in comparison to control patients (Zeis et al., 2008; Zeis et al., 2009). Furthermore, we demonstrated that in the NAWM, STAT6 is expressed mainly by oligodendrocytes and to a minor extent in astrocytes (Zeis et al., 2008). Interestingly, we found that STAT6, as well as two members of the STAT6 pathway, IL4 and IL4R, are higher expressed in AL, RL and IdL in comparison to NAWM. This suggests that a possible protective, anti-inflammatory reaction already activated in the NAWM, is intensified in zones of ongoing inflammation in MS, although other non-immunological related functions cannot be ruled out (Zeis et al., 2016).

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Pathway analysis of the differential gene expression between the different tissues is particularly difficult due to the highly variable cellular composition (Figure 1). This might result in a different RNA pool and, therefore, the resulting differential gene expression is predominantly defined by the change in cell type composition and changes in gene expression of resident cells might be masked. This is reflected by the results of the biological function analysis, which showed that the most enriched processes detected were related to the immune system (Table 6). However, in addition to that, we found a signature of an increased *development of neurons*, *cell viability of CNS cells* and *axonogenesis* in AL and *myelination* in RL tissue when compared with NAWM tissue. These signatures suggest that glial cells react to the inflammation with a protective response and/or infiltrating immune cells support the survival of resident cells by expressing genes supportive of cell viability.

Understanding the signaling pathways leading to lesion remyelination in MS is key for devising therapies that enhance repair. Our study was designed to identify common factors that might distinguish the different tissue types (NAWM, AL, RL and IdL) and serve as therapeutic candidates to enhance remyelination. In summary, several different factors known to be involved in oligodendrocyte development were detected to be differentially regulated between the different tissue types (summarized in Figure 5). However, no specific de- or remyelinating pattern was detected in either tissue type; rather a largely heterogeneous expression pattern of oligodendrogliogenesis influencing genes was detected between, but also within, the different lesion types. This indicates that at any one point in time in the MS brain, developing and repairing lesions can be present at many different stages, in keeping with a continuum of ongoing processes. A greater understanding of this heterogeneity is crucial if we are to understand the correct timing for delivery of agents that enhance remyelination. Therefore, a precise characterization of the temporal and spatial localization of pro-repair factors is required.

Author contribution

N.S-W. contributed to study design, oversight and manuscript preparation. R.R. contributed to study design, oversight and sample selection. O.H. contributed to

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sample selection and characterisation, RNA isolation and array hybridization. T.Z. performed the data analysis and interpretation, qPCR and manuscript preparation.

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Figure and Table Legends

Figure 1: Tissue characterization

All tissue blocks were stained with LFB (A, E, I, J), anti-HLA-DR (LN3, A, B, D, E, H, K, M) and anti-MOG (C, L) antibodies. Accordingly, tissue was characterized and subsequent sections selected for the micro-dissection of tissue for total RNA isolation. Normal appearing white matter tissue showed normal, non-pathological myelin (A) and microglia with a surveilant and ramified morphology (A, B). In contrast, active lesions showed a loss of myelin (C) as well as activated microglia/macrophages containing myelin lipids (D, E). Remyelinating tissue was either characterized as partially remyelinated lesions containing MOG+ oligodendrocytes and short myelin internodes in areas of ongoing inflammation (F, G and H) termed as active RL (aRL) or completely remyelinated (cRL) and characterized by the pale myelin staining (LFB) of the shadow plaque (I, J) accompanied by modest numbers of activated microglia/ macrophages (K). Inactive demyelinated tissue showed a complete demyelination (L) without significant active inflammation or myelin debris (M). Left panels: Schematic drawing of MS lesions show the simplified cellular situation in the specified tissue in order to visualize the cellularity defining the mRNA isolated. Oligodendrocytes and their myelin sheets (brown), astrocytes (green), microglia (yellow) as well as activated microglia / macrophage (grey) and other infiltrating immune cells of various types (blue) are shown. Asterisks = lesion tissue

Figure 2: Differential gene expression between the different tissues

Volcano plots of the differential gene expression in between the different tissues. All possible comparisons are shown. However, for better visibility, only a selection of gene names is shown. All genes with a p-value of less than 0.05 and a fold change of +/- 1.5 are marked by red or green color, respectively. Most significantly differential expressed genes were found between NAWM and IdL whereas the AL and RL tissues seemed to be the most similar.

Figure 3: Cluster analysis

Cluster analysis of the gene expression data from all samples reveals three main clusters. Cluster 1 contained most of the NAWM tissue (6 out of 9) whereas in cluster

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2 most IdL tissues were found. AL and RL tissues were dispersed in all three clusters. Clusters were not formed by possible confounding factors such as patient or batch.

Figure 4: Boxplots of selected genes

Boxplots of differential gene expression between NAWM, AL, RL and IdL of selected genes are shown. A high variability in between samples of the same tissue type is visible for different genes, such as e.g. IGF1 (A), NKX2-2 (D, AL & IdL) and SEMA3B (F, AL). Whereas IGF1 (A) and IGF2 (B) expression shows no clear trend between the different tissue types, the expression of CXCL12 (C) is increased from NAWM to IdL lesion tissues. In contrast, the expression of NKX2-2 (D), SOX10 (E) and SEMA3B (F) are decreased from NAWM to IdL tissue. The expression of STAT6 (G) and IL4 (H) tend to be the highest in RL tissue, whereas the expression of IL4R (I) is highest in IdL. As expected, the expression of the myelin genes MAL (J), MOG (K) and PLP1 (M) is lowest in IdL tissues.

Figure 5: Schematic drawing summarising the differential expression pattern of genes involved in oligodendrogliogenesis

Summary scheme showing significantly differentially expressed genes involved in oligodendrogliogenesis between the different tissue types (Table 4). Genes are color coded according to their higher (green) or lower (red) expression in the tissue the color matching arrow points to.

Table 1: Patient characterization

Summary of clinical data and characterization of MS cases. Clinical and pathological information concerning the 7 progressive MS cases studied are shown. NAWM, normal appearing white matter, AL, active lesion, RL, remyelinating lesion, IdL, inactive demyelinated lesion.

Table 2: Antibodies

Antibodies used in this study. Table shows all antibodies, their origin and dilution used. Abbreviations: dk-a-rb, donkey anti rabbit; dk-a-m, donkey anti mouse; dk-a-gt, donkey anti goat.

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Table 3: Top genes showing most significant changes between the different MS tissue types

30 genes with most significant changes in gene expression between the different tissue types is shown. Genes with a p-value of less than 0.05 are marked by a grey background color and were further colored according to their fold change. Genes with a fold change higher than 1.5 were marked red. Genes with a fold change lower than -1.5 were marked green.

Table 4: Differential expression of genes involved in oligodendrogenesis

Differential gene expression of genes known to be involved in oligodendrogenesis. Differential gene expression was analysed between all four different tissue types (AL, RL, IdL and NAWM). Table shows relative fold changes in between the different tissue types of a selection of genes known to be involved in oligodendrogenesis. Genes with a p-value of less than 0.05 are shown in bold, and were colored according to their fold change. Genes with a fold change higher than 1.5 were marked red. Genes with a fold change lower than -1.5 were marked green.

Table 5: Differential expression of STAT6 pathway genes

Differential gene expression of genes of the STAT6 pathway. Relative fold changes are shown. Differential expression was analysed between AL, RL and IdL in comparison to NAWM. Statistically significant changes are marked in bold red.

Table 6: Ingenuity pathway analysis

Ingenuity pathway analysis of the differential gene expression between NAWM, AL, RL and IdL. Differential gene expression between NAWM, AL, RL and IdL was analysed to predict the increase/decrease of activation of particular functions in the different tissues. A selection of functions predicted to be activated/inhibited is shown.

Table 7: qRT-PCR verification

To verify the results from the ATLAS cDNA gene expression array we performed a quantitative RT-PCR analysis of selected genes. In a majority of comparisons the results from the ATLAS cDNA expression array matched with the qRT-PCR analysis.

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Supplementary table 1: Differential gene expression analysis

Table shows the fold change and p-value of all genes and comparisons investigating in this study.

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Figure 1: Tissue characterization

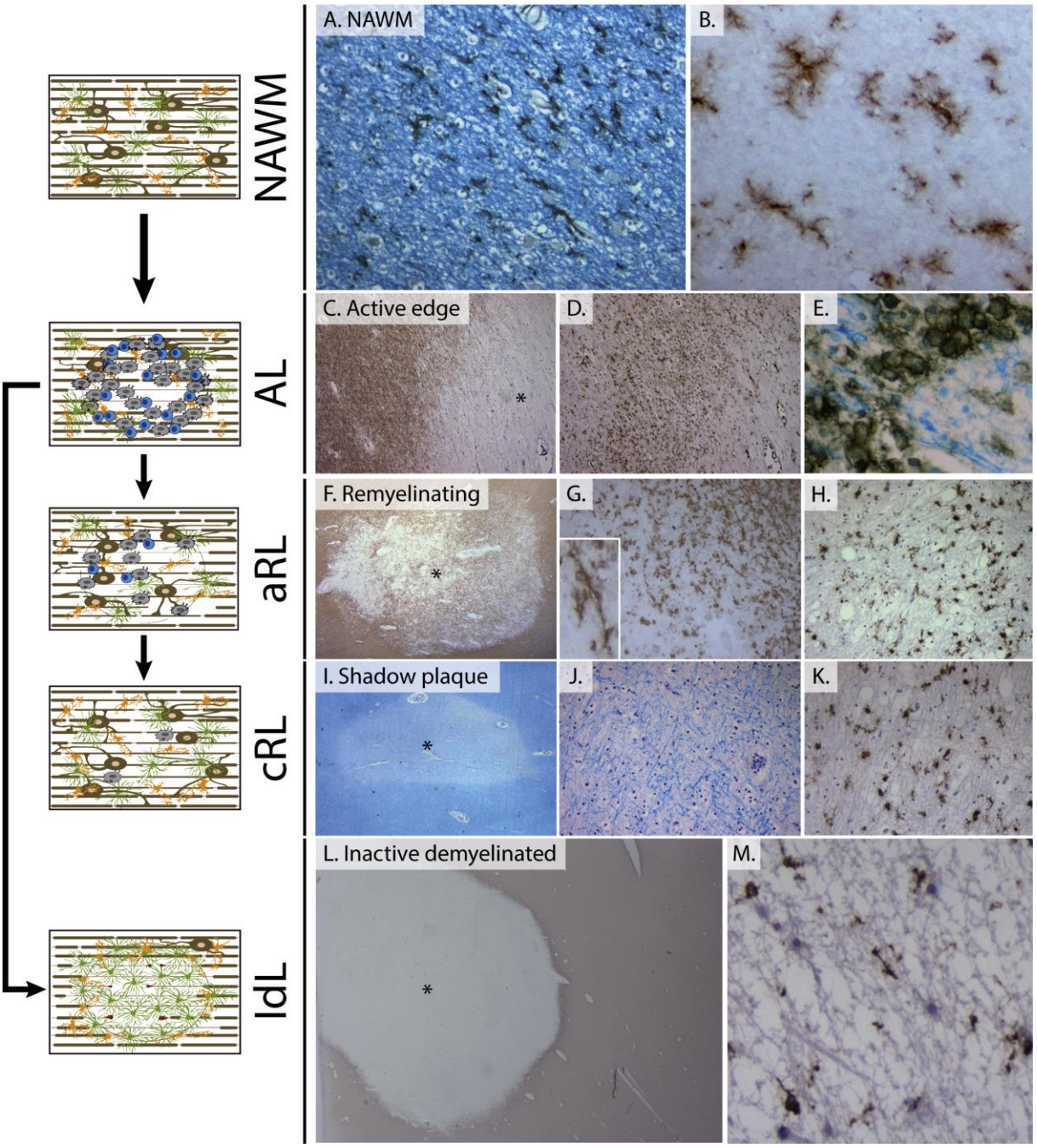

Figure 2:

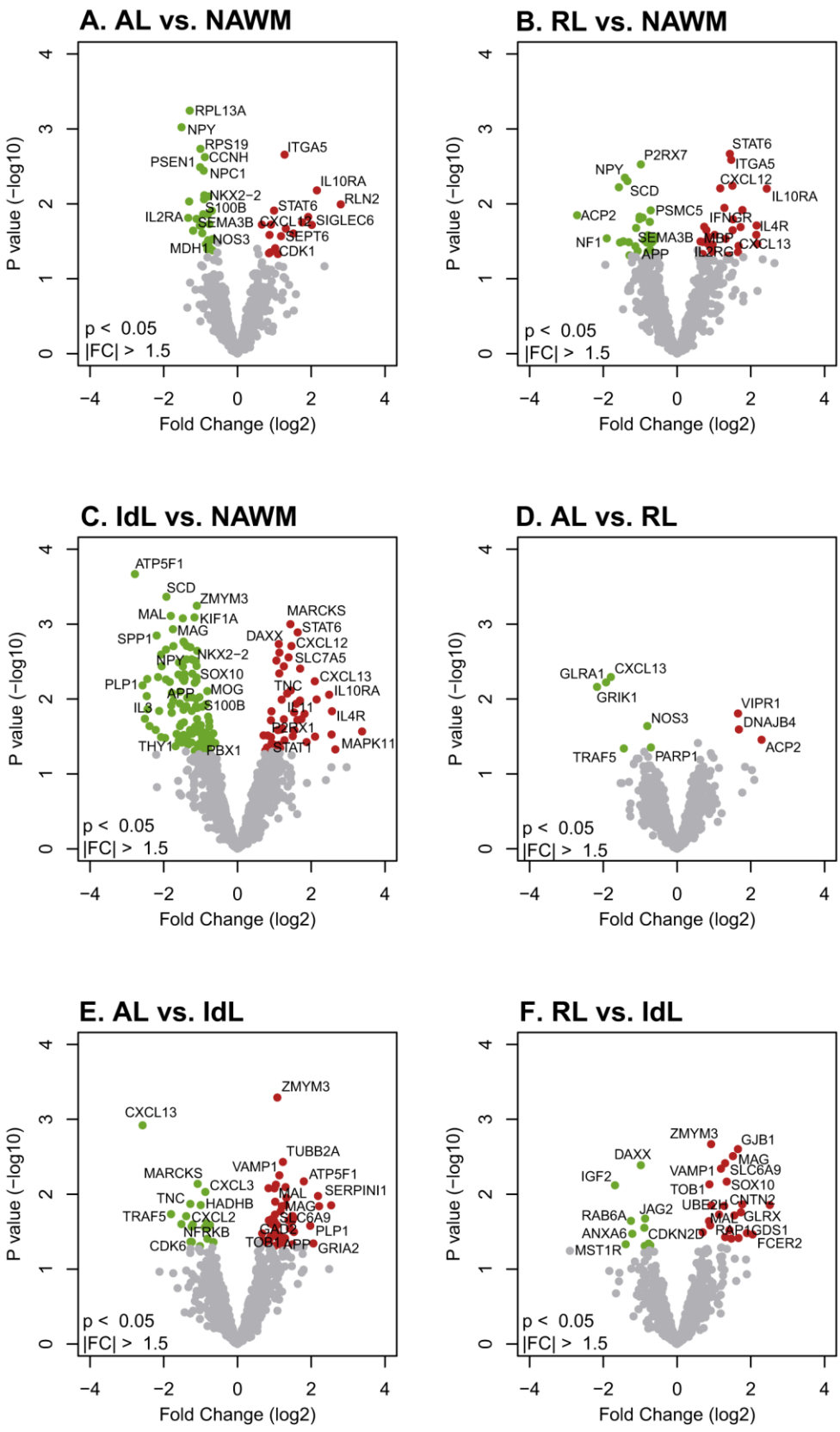
A. AL vs. NAWM
RPL13A
NPY
RPS19
CCNH
PSEN1
NPC1
ITGA5
IL10RA
RLN2
NKX2−2
S100B
IL2RA
SEMA3B
CXCL12
STAT6
SIGLEC6
NOS3
SEPT6
MDH1
CDK1
p < 0.05
|FC| > 1.5
P value (−log10)
Fold Change (log2)
B. RL vs. NAWM
STAT6
P2RX7
ITGA5
NPY
CXCL12
SCD
IL10RA
ACP2
PSMC5
IFNGR
IL4R
NF1
SEMA3B
MBP
CXCL13
APP
IL2RG
p < 0.05
|FC| > 1.5
P value (−log10)
Fold Change (log2)
C. IdL vs. NAWM
ATP5F1
SCD
ZMYM3
MAL
KIF1A
MARCKS
MAG
DAXX
STAT6
SPP1
CXCL12
NKX2−2
SLC7A5
NPY
SOX10
CXCL13
PLP1
TNC
MOG
IL10RA
APP
S100B
IL1
IL4R
P2RX1
THY1
PBX1
STAT1
MAPK11
p < 0.05
|FC| > 1.5
P value (−log10)
Fold Change (log2)
D. AL vs. RL
GLRA1
CXCL13
GRIK1
VIPR1
NOS3
DNAJB4
ACP2
TRAF5
PARP1
p < 0.05
|FC| > 1.5
P value (−log10)
Fold Change (log2)
E. AL vs. IdL
ZMYM3
CXCL13
TUBB2A
VAMP1
MARCKS
ATP5F1
CXCL3
MAL
SERPINI1
TNC
HADHB
MAG
TRAF5
CXCL2
SLC6A9
NFKB
PLP1
CDK6
TOB1
APP
GRIA2
p < 0.05
|FC| > 1.5
P value (−log10)
Fold Change (log2)
F. RL vs. IdL
ZMYM3
GJB1
DAXX
MAG
IGF2
VAMP1
SLC6A9
TOB1
SOX10
CNTN2
RAB6A
JAG2
MAL
GLRX
ANXA6
CDKN2D
RAP1GDS1
MST1R
FCER2
p < 0.05
|FC| > 1.5
P value (−log10)
Fold Change (log2)

Figure 3:

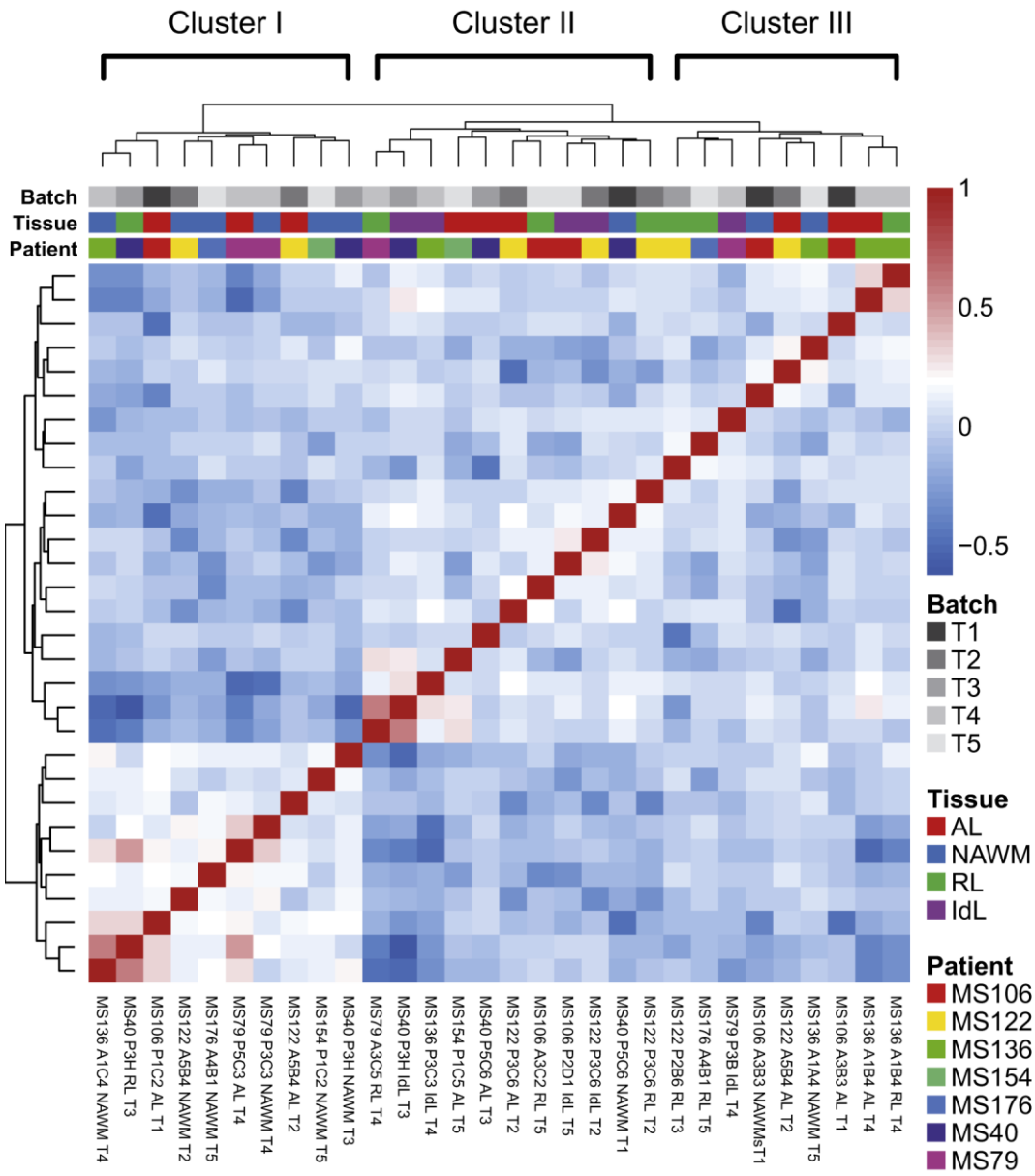
Cluster I
Cluster II
Cluster III
Batch
Tissue
Patient
1
0.5
0
−0.5
Batch
T1
T2
T3
T4
T5
Tissue
AL
NAWM
RL
IdL
Patient
MS106
MS122
MS136
MS154
MS176
MS40
MS79
MS136 A1C4 NAWM T4
MS40 P3H RL T3
MS106 P1C2 AL T1
MS122 A5B4 NAWM T2
MS176 A4B1 NAWM T5
MS79 P5C3 AL T4
MS79 P3C3 NAWM T4
MS122 A5B4 AL T2
MS154 P1C2 NAWM T5
MS40 P3H NAWM T3
MS79 A3C5 RL T4
MS40 P3H IdL T3
MS136 P3C3 IdL T4
MS154 P1C5 AL T5
MS40 P5C6 AL T3
MS122 P3C6 AL T2
MS106 A3C2 RL T5
MS106 P2D1 IdL T5
MS122 P3C6 IdL T2
MS40 P5C6 NAWM T1
MS122 P3C6 RL T2
MS122 P2B6 RL T3
MS176 A4B1 RL T5
MS79 P3B IdL T4
MS106 A3B3 NAWMsT1
MS122 A5B4 AL T2
MS136 A1A4 NAWM T5
MS106 A3B3 AL T1
MS136 A1B4 AL T4
MS136 A1B4 RL T4

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Figure 4: Boxplots of selected genes

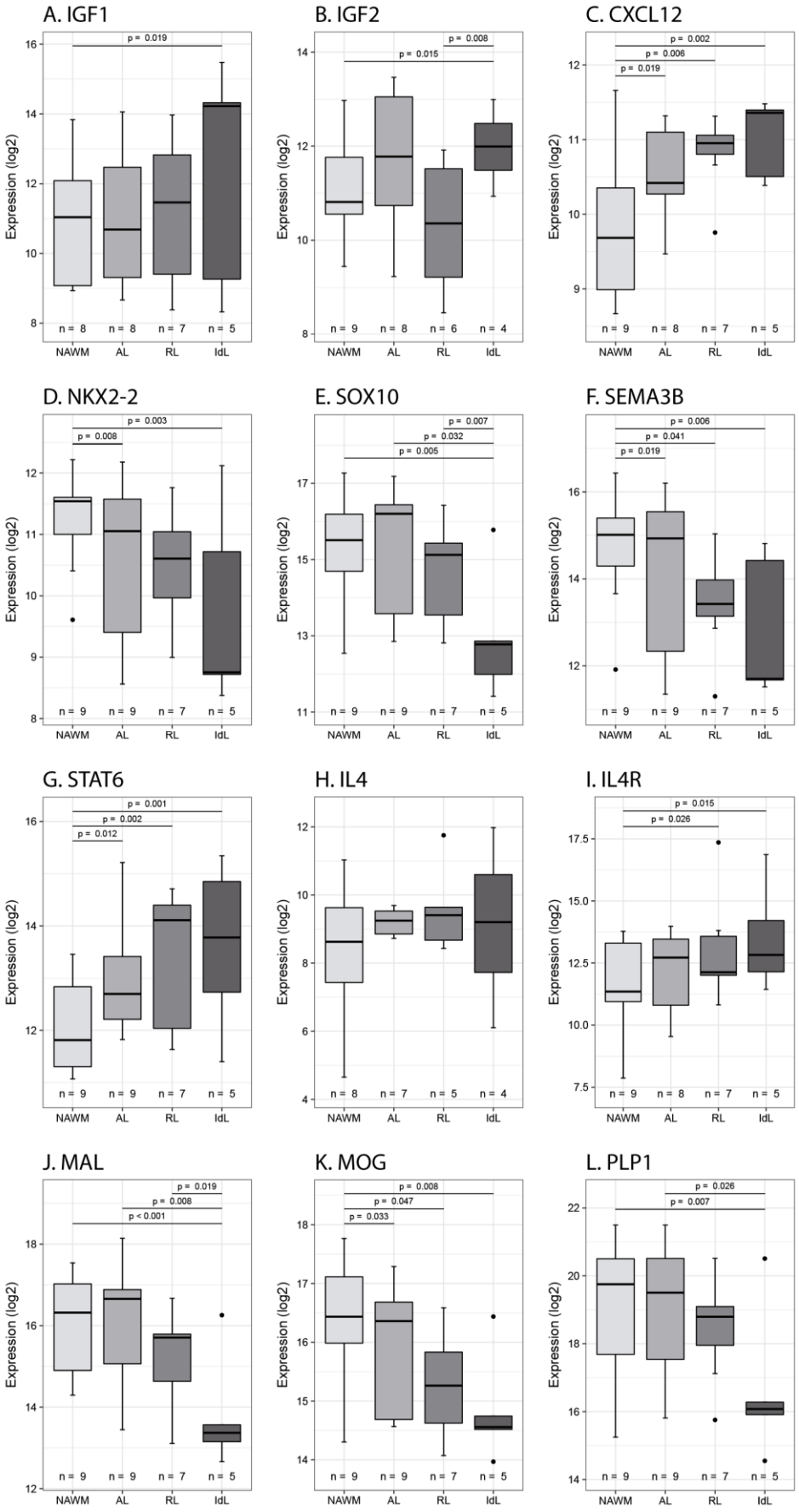
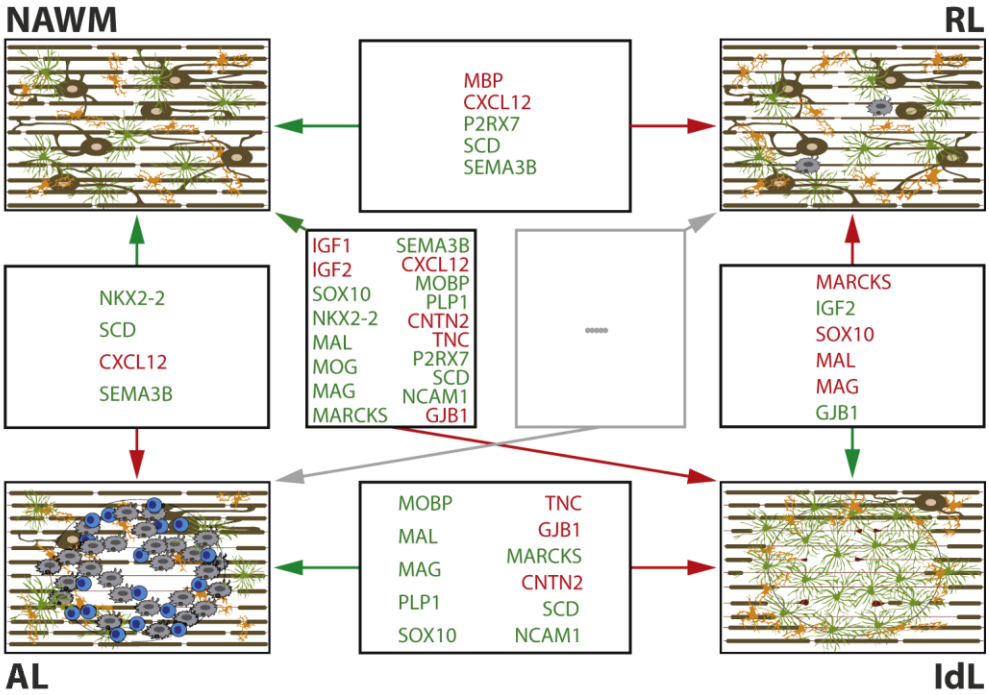


Figure 5: Schematic drawing summarising the differential expression pattern of genes involved in oligodendrogliogenesis



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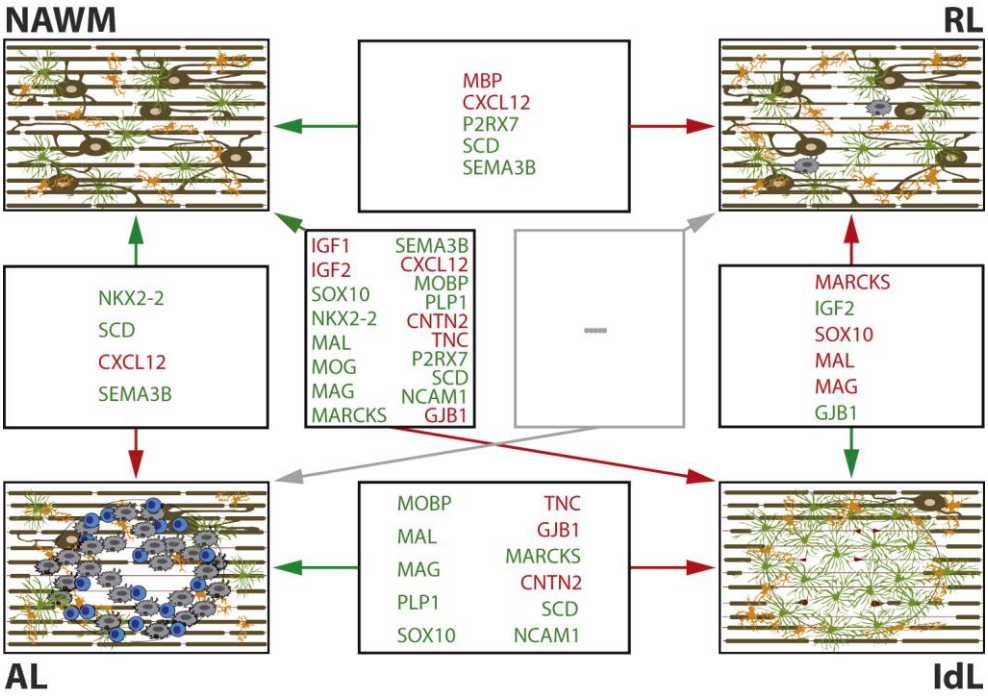

Graphical Abstract

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Table 1

Patient ID	Age (yr)	Sex	MS Type	Disease Duration (yrs)	Postmortem Interval (hrs)	Cause of Death	NAWM	AL	RL	IdL
MS40	48	F	PP	21	6	Bronchopneumonia	2	1	1	1
MS79	49	F	SP	23	7	Bronchopneumonia	1	1	1	1
MS106	39	F	PP	21	18	Bronchopneumonia	1	2	1	1
MS122	44	M	SP	16	16	Bronchopneumonia	1	3	2	1
MS136	40	M	SP	9	10	Respiratory failure, sepsis	2	1	1	1
MS154	34	F	SP	11	12	Pneumonia	1	1	-	-
MS176	37	M	PP	27	12	Intestinal obstruction	1	-	1	-

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Table 2: Antibodies

Antibody	Company	Cat.Nr.	Dilution
Primary Antibodies			
anti-MOG (Clone Z12)	kindly provided by Prof. R.Reynolds	-	1:200
anti-NEUN	Millipore	MAB377	1:500
anti-GFAP	Sigma-Aldrich	G-3893	1:2000
anti-CD68	Abcam	ab845	1:500
anti-LN3	Abcam	ab49388	1:500
anti-OLIG2	Millipore	ab9610	1:500
Secondary Antibodies			
dk-a-rb-Biotin	Jackson ImmunoResearch	711-065-152	1:500
dk-a-m-Biotin	Jackson ImmunoResearch	715-065-151	1:500
dk-a-gt-Biotin	Jackson ImmunoResearch	705-065-147	1:500

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Table 3

		AL			RL		IdL
		NAWM	RL	IdL	NAWM	IdL	NAWM
SYMBOL	Gen name	FC	FC	FC	FC	FC	FC
CXCL13	chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)	-1.39	**-3.47**	**-5.95**	**2.50**	**-1.71**	**4.28**
PARP1	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)	-1.01	**-1.64**	**-1.78**	**1.62**	-1.09	**1.77**
PEA15	astrocytic phosphoprotein PEA-15	-1.03	1.48	1.48	**-1.52**	-1.00	**-1.52**
SCD	ACYL-COA DESATURASE (EC 1.14.99.5) (STEAROYL-COA DESATURASE) (FATTY ACID DESATURASE) (DELTA(9)-DESATURASE).	**-1.81**	1.40	**2.10**	**-2.54**	1.50	**-3.80**
ACVRL1	activin A receptor type II-like 1	**4.04**	-1.10	-1.06	**4.46**	1.04	**4.30**
ALCAM	CD166 ANTIGEN PRECURSOR (ACTIVATED LEUKOCYTE-CELL ADHESION MOLECULE) (ALCAM).	**-1.51**	1.13	**2.78**	**-1.70**	**2.47**	**-4.20**
APP	Alzheimer's disease amyloid A4 protein precursor; protease nexin-II (PN-II); APPI	-1.33	**1.58**	**2.23**	**-2.10**	1.41	**-2.95**
ATP5F1	ATP SYNTHASE B CHAIN, MITOCHONDRIAL PRECURSOR	**-1.98**	1.19	**3.48**	**-2.35**	**2.93**	**-6.88**
CHRNA6	cholinergic receptor, nicotinic, alpha polypeptide 6	**-2.30**	**-2.26**	**1.51**	-1.02	**3.41**	**-3.48**
COX5B	CYTOCHROME C OXIDASE POLYPEPTIDE VB PRECURSOR (EC 1.9.3.1).	**-1.62**	1.03	1.07	**-1.67**	1.04	**-1.74**
CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	**1.88**	-1.20	-1.47	**2.25**	-1.22	**2.76**
DAXX	death-associated protein 6	1.38	1.26	**-1.57**	1.10	**-1.98**	**2.17**
DYNLL1	cytoplasmic dynein light chain 1 (HDLC1); protein inihibitor of neuronal nitric oxide synthase (PIN)	**-1.88**	1.02	1.49	**-1.91**	1.46	**-2.79**
EDIL3	INTEGRIN BINDING PROTEIN DEL-1.	**-1.54**	1.04	**2.89**	**-1.61**	**2.77**	**-4.45**
ELAVL3	HU-ANTIGEN C (PARANEOPLASTIC CEREBELLAR DEGENERATION-ASSOCIATED ANTIGEN).	-1.18	1.13	**2.06**	-1.33	**1.82**	**-2.43**
FSCN1	FASCIN (ACTIN BUNDLING PROTEIN).	-1.14	**1.52**	**2.00**	**-1.73**	1.32	**-2.28**
GJB1	GAP JUNCTION BETA-1 PROTEIN (CONNEXIN 32) (CX32) (LIVER GAP JUNCTION PROTEIN)	-1.33	-1.25	**2.51**	-1.06	**3.14**	**-3.34**
GSTO1	glutathione-S-transferase like; glutathione transferase omega	**-1.90**	1.07	1.02	**-2.03**	-1.04	**-1.95**
IL10RA	interleukin 10 receptor, alpha	**4.45**	-1.21	-1.26	**5.40**	-1.04	**5.60**
IL2RA	interleukin 2 receptor, alpha	**-2.53**	-1.02	1.00	**-2.48**	1.02	**-2.53**
ITGA5	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	**2.42**	-1.14	-1.05	**2.77**	1.09	**2.54**
ITSN1	intersectin 1 (SH3 domain protein)	**1.58**	-1.05	-1.31	**1.67**	-1.25	**2.08**

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JAG2	jagged 2	1.07	1.04	-1.76	1.03	-1.82	1.88
KCNJ2	cardiac inward rectifying potassium channel 2 (KCNJ2); HIRK1; KIR2.1	-1.72	-1.07	1.27	-1.61	1.36	-2.19
KIF1A	kinesin-like protein KIF1A; axonal transporter of synaptic vesicles	-1.25	1.24	1.79	-1.56	1.44	-2.25
MAG	myelin-associated glycoprotein precursor (MAG)	-1.46	-1.24	2.30	-1.18	2.86	-3.37
MAL	mal, T-cell differentiation protein	-1.42	1.12	2.47	-1.59	2.20	-3.50
MARCKS	myristoylated alanine-rich C-kinase substrate (MARCKS); protein kinase C substrate 80-kDa protein light chain (PKCSL)	1.28	-1.24	-2.11	1.58	-1.71	2.70
MOG	myelin-oligodendrocyte glycoprotein precursor (MOG)	-1.44	1.01	1.23	-1.46	1.21	-1.76
NPY	neuropeptide Y	-2.86	-1.07	1.05	-2.67	1.13	-3.00
PIP4K2A	phosphatidylinositol-4-phosphate 5-kinase type III (PIP5K3); PTDINS(4)P-5-kinase C isoform; diphosphoinositide kinase + phosphatidylinositol-4-phosphate 5-kinase type II alpha (PIP5K2A); PTDINS(4)P-5-kinase B isoform	-1.45	1.07	2.87	-1.56	2.68	-4.16

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Table 4:

Symbol	Gene name	Relative fold changes AL vs. NAWM	RL vs. NAWM	IDL vs. NAWM	RL vs. AL	IDL vs. AL	RL vs. IDL
	Growth factors						
BMP2	bone morphogenetic protein 2	1.10	1.18	1.17	1.07	1.07	0.99
FGF2	fibroblast growth factor 2	1.52	1.20	-1.08	-1.26	-1.64	1.30
FGF9	fibroblast growth factor 9	1.18	1.30	1.22	1.11	1.04	1.07
IGF1	insulin-like growth factor 1	1.71	1.69	**3.33**	-1.01	1.95	-1.97
IGF2	insulin-like growth factor 2	1.54	-1.11	**2.90**	-1.71	1.88	**-3.21**
JAG1	jagged 1	1.25	1.57	1.93	1.26	1.54	-1.23
NTF3	neurotrophin 3	1.05	-1.30	-1.17	-1.37	-1.23	-1.11
PDGFA	plateled-derived growth factor, alpha polypeptide	-1.11	1.04	1.02	1.15	1.13	1.02
	Receptors						
CNTFR	ciliary neurotrophic factor receptor	1.26	1.08	1.48	-1.16	1.18	-1.37
EGFR	epidermal growth factor receptor	1.29	1.31	-1.03	1.01	-1.33	1.34
FGFR1	fibroblast growth factor receptor 1	1.16	1.43	1.38	1.24	1.20	1.04
FGFR2	fibroblast growth factor receptor 2	-1.10	1.27	1.12	1.40	1.23	1.14
FGFR3	fibroblast growth factor receptor 3	-1.00	1.32	1.60	1.32	1.60	-1.21
FGFR4	fibroblast growth factor receptor 4	1.36	2.16	1.68	1.59	1.24	1.29
LIFR	leukemia inhibitory factor receptor alpha	-1.72	-2.17	-1.65	-1.26	1.04	-1.31
NGFR	nerve growth factor receptor	-1.01	2.04	2.33	2.06	2.36	-1.14
NRP1	neuropilin 1	-1.04	1.12	1.05	1.17	1.09	1.07
NRP2	neuropilin 2	-1.07	1.00	1.33	1.07	1.42	-1.33
SEMA4D	semaphorin 4D	1.08	1.27	1.08	1.17	-1.00	1.17
P2RX7	purinergic receptor P2X 7	-1.43	**-1.98**	**-2.13**	-1.38	-1.49	1.08
	Transcription factors						

NOTCH1	Notch1	-1.06	-1.38	-1.07	-1.30	-1.01	-1.29
NKX2-2	NK2 homeobox 2	**-1.87**	-1.49	**-2.37**	1.25	-1.27	1.59
SOX2	SRY-box 2	1.08	1.40	1.47	1.30	1.36	-1.05
SOX9	SRY-box 9	-1.03	1.08	-1.10	1.11	-1.07	1.19
SOX10	SRY-box 10	-1.32	-1.06	**-2.70**	1.25	**-2.04**	**2.54**
	Myelin genes						
MAG	myelin-associated glycoprotein	-1.46	-1.18	**-3.37**	1.24	**-2.30**	**2.86**
MAL	myelin and lymphocyte protein	-1.42	-1.59	**-3.50**	-1.12	**-2.47**	**2.20**
MBP	myelin basic protein	1.31	**1.81**	1.68	1.38	1.28	1.07
MOBP	myelin-associated oligodendrocytic basic protein	-1.43	-1.71	**-3.83**	-1.20	**-2.68**	2.24
MOG	myelin oligodendrocyte glycoprotein	-1.44	-1.46	**-1.76**	-1.01	-1.23	1.21
PLP1	proteolipid protein 1	-1.52	-2.01	**-5.96**	-1.33	**-3.93**	2.96
SCD	stearoyl-CoA desaturase	**-1.81**	**-2.54**	**-3.80**	-1.40	**-2.10**	1.50
	Cytokines						
CXCL12	chemokine (C-X-C motif) ligand 12	**1.88**	**2.25**	**2.76**	1.20	1.47	-1.22
IL6	interleukin 6	1.13	-1.05	-1.17	-1.18	-1.32	1.12
LIF	leukemia inhibitory factor	-1.61	-1.03	-1.22	1.57	1.33	1.18
	Others						
CSPG4	chondroitin sulfate proteoglycan 4	-1.11	1.23	1.04	1.37	1.16	1.18
TH	tyrosine hydroxylase	-1.11	1.24	1.24	1.38	1.37	1.01
PTPN11	protein tyrosine phosphatase, non-receptor type 11	-1.09	-1.46	-1.68	-1.34	-1.54	1.15
SEMA3B	semaphorin 3B	**-1.69**	**-1.66**	**-2.16**	1.02	-1.28	1.30
RELN	reelin	1.01	-1.06	-1.19	-1.08	-1.21	1.12
NCAM1	neural cell adhesion molecule 1	-1.07	-1.54	**-2.14**	-1.44	**-2.00**	1.39
VTN	vitronectin	-1.21	-1.04	-1.47	1.17	-1.22	1.42

TNC	tenascin C	1.12	1.89	**2.72**	1.69	**2.43**	-1.44
THRB	thyroid hormone receptor, beta	-1.48	1.00	-1.10	1.49	1.35	1.10
MARCKS	myristoylated alanine rich protein kinase C substrate	1.28	-1.24	**-2.11**	1.58	**-1.71**	**2.70**
GJB1	gap junction protein beta 1	-1.33	-1.25	**2.51**	-1.06	**3.14**	**-3.34**
CNTN2	contactin 2	-1.20	-1.60	1.51	1.34	**2.24**	-1.81

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Table 5:

	Gene	NAWM	AL	RL	IdL
STAT6 Pathway	STAT6	1	**1.99**	**2.7**	**3.1**
	JAK1	1	1.1	0.83	1.12
	JAK3	1	0.67	0.76	0.88
	IL4	1	2.07	**3.47**	1.99
	IL4R	1	2.35	**4.43**	**5.9**

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Table 6:

Biological Function Annotation	p-Value	Predicted activation	Activation z-score
AL vs NAWM			
Development of neurons	2.08E-12	Increased	2.672
Cell viability of central nervous system cells	6.95E-12	Increased	2.478
Cell viability of neuroglia	3.89E-13	Increased	2.26
Cell viability of myeloid cells	2.79E-13	Increased	2.138
Stimulation of myeloid cells	1.31E-12	Increased	2.046
Axonogenesis	2.33E-11	Increased	2.019
RL vs NAWM			
Leukocyte migration	8.34E-37	Increased	2.651
Development of neurons	3.23E-14	Increased	2.616
Cell proliferation of T lymphocytes	4.49E-21	Increased	2.456
Proliferation of immune cells	1.71E-22	Increased	2.296
Proliferation of neuronal cells	4.42E-15	Increased	2.257
Myelination	3.28E-15	Increased	2.17
IdL vs NAWM			
Proliferation of lymphocytes	2.86E-34	Increased	2.728
Proliferation of mononuclear leukocytes	4.67E-36	Increased	2.672
Proliferation of immune cells	1.87E-37	Increased	2.434
T cell migration	1.15E-19	Increased	2.14
AL vs RL			
Cell movement of phagocytes	6.26E-27	Decreased	-3.419
Leukocyte migration	1.07E-31	Decreased	-2.768
Cellular infiltration by macrophages	2.38E-13	Decreased	-2.692
T cell response	3.40E-10	Decreased	-2.33
Proliferation of immune cells	3.10E-18	Decreased	-2.294
AL vs IdL			
Inflammation of organ	1.29E-39	Increased	2.748
Viral Infection	9.85E-26	Increased	2.308
Cell proliferation of tumor cell lines	1.96E-28	Increased	2.19
RL vs IdL			
Systemic autoimmune syndrome	7.03E-19	Increased	2.672

Inflammation of organ	1.17E-23	Increased	2.522
Stimulation of T lymphocytes	1.74E-08	Increased	2.319
Stimulation of lymphocytes	1.18E-09	Increased	2.211
Chemotaxis of granulocytes	5.04E-09	Increased	2.154

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Table 7:

Gene	AL vs NAWM		RM vs NAWM		IdL vs NAWM	
	Array	qPCR	Array	qPCR	Array	qPCR
MAL	-1.42	**-1.93**	-1.59	**-1.80**	**-3.50**	**-3.85**
MAG	-1.46	-1.47	-1.18	-1.38	**-3.37**	**-2.83**
IL4R	2.35	1.31	**4.43**	1.18	**5.9**	1.43
STAT6	**1.99**	**1.58**	**2.7**	1.16	**3.1**	**1.71**
CXCL12	**1.88**	**1.92**	**2.25**	**2.02**	**2.76**	**3.56**
IGF2	1.54	1.22	-1.11	1.25	**2.90**	**2.22**

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Highlights

- Lesion tissues express both, oligodendrogliogenesis promoting and inhibiting genes
- Gene expression patterns vary significantly in single lesion tissue types
- Gene expression heterogeneity might be causative for non-uniform remyelination

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