Supplemental Materials

Signatures for Viral Infection and Inflammation in the Proximal Olfactory System in Familial Alzheimer's Disease

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eNote 1. Annotations regarding the function of proteins in groups A and B of the nanoString (Fig. 3Dii)

nanoString group A proteins: high expression in FAD high myelination OT (Fig. 3Dii)

The majority of these proteins are involved in demyelination or are axonal proteins.

Neurofilament light. Neurofilament light chain (NfL) is a neuronal cytoplasmic protein highly expressed in large calibre myelinated axons (Gaetani et al., 2019).

Fibronectin (FN1). In toxin-induced lesions undergoing efficient remyelination, fibronectin expression was transiently increased within demyelinated areas and declined as remyelination proceeded(Stoffels et al., 2013).

INPP4B. EAE31 is a locus controlling latency of motor evoked potentials (MEPs) and clinical onset of experimental autoimmune encephalomyelitis. By combining congenic mapping, *in silico* haplotype analyses, and comparative genomics Lemcke and co-workers identified inositol polyphosphate-4-phosphatase, type II (*Inpp4b*) as the quantitative trait gene for EAE31(Lemcke et al., 2014).

CD25 (IL2RA). Depletion of $CD4^+CD25^+$ cells *in vivo* facilitated the expansion of PLP reactive cells with production of T helper 1 cytokines in EAE-resistant B10.S mice. Furthermore, anti-CD25 Ab treatment before immunization resulted in EAE induction in these otherwise resistant mice. These data indicate an important role for autoantigen-specific $CD4^+CD25^+$ cells in genetic resistance to autoimmunity(Reddy et al., 2004).

Ubiquitin. The vast majority of cellular proteins are degraded by the 26S proteasome after their ubiquitination. Belogurov et al. (Belogurov et al., 2014) report that the major component of the myelin multilayered membrane sheath, myelin basic protein (MBP), is hydrolyzed by the 26S proteasome in a ubiquitin-independent manner both *in vitro* and in mammalian cells(Belogurov et al., 2014).

PD.L2. Programed death ligand 2. PD-L2 is up-regulated in inflamed endothelial cells, with an intention to inhibit T-cell transmigration through the blood brain barrier(Zhao et al., 2014).

ICOS. The inducible costimulatory molecule (ICOS) is expressed on activated T cells and participates in a variety of important immunoregulatory functions. After the induction of experimental allergic encephalomyelitis in SJL mice with proteolipid protein (PLP), brain ICOS mRNA and protein were up-regulated on infiltrating CD3+ T cells before disease onset.(Rottman et al., 2001)

CD80. CD4+ T Cell Expressed CD80 Regulates Central Nervous System Effector Function and Survival during Experimental Autoimmune Encephalomyelitis(Podojil et al., 2006).

PD.1 and PD.L1. The review by Cencioni et al describes the roles of the PD-1/ PDL-1 pathway in cancer and autoimmune diseases, especially in multiple sclerosis, and how manipulating PD-1 can be a therapeutic approach in multiple sclerosis(Cencioni, 2020).

CD44. CD44 overexpression is thought to cause inflammation-independent demyelination and dysmyelination(Tuohy et al., 2004).

Phospho-PRAS40..T246. PRAS40 (Proline-rich AKT1 substrate 1), also known as Akt1S1 and p39, is a 40-42 kDa cytoplasmic phosphoprotein that lacks generally recognized structural motifs. It is widely expressed and is considered to be key regulator of mTORC1 (mTOR plus Raptor and G beta L), a complex through which Akt signals into the cell. Through phosphorylation, mTORC1 activity is upregulated by PRAS40(Bercury et al., 2014).

Phospho-AKT1..S473. Involved in the dual function of the PI3K-Akt-mTORC1 axis in myelination of the peripheral nervous system(Figlia et al., 2017).

Phsopho-GSK3B.S9. MAI-dependent phosphorylation and inactivation of GSK3beta regulate phosphorylation of CRMP4, a cytosolic regulator of myelin inhibition, and its ability to complex with RhoA(Alabed et al., 2010).

Vimentin. Increase in tissue stiffness elicited by chronic demyelination of the corpus callosum is accompanied by astrogliosis, as shown by elevated GFAP and vimentin staining(Urbanski et al., 2019).

CD68. This is a marker for macrophages. Myelin loss along with axonal destruction, the pathological hallmark of metachromatic leukodystrophy is thought to be caused by critical sulphatide levels in oligodendrocytes and Schwann cells. Immunolabelling with MBP and CD68 showed a gradient of demyelination from near-intact U-fibres to myelin-depleted white matter with diffuse macrophage infiltration.(Ponath et al., 2017).

APP. Data by Truong et al. identified APP and APLP2 as modulators of normal myelination and demyelination/remyelination conditions. Deletion of APP and APLP2 identifies novel interplays between the BACE1 substrates in the regulation of myelination(Truong et al., 2019).

Myelin basic protein. MBP is a protein found in the myelin sheath.

CD45RO. Dual expression of CD45RA and CD45RO isoforms on myelin basic protein-specific CD4+ T-cell lines in multiple sclerosis(Qin et al., 1993)

nanoString group B proteins: high expression in FAD low myelination OT (Fig. 3Dii)

These proteins are AD markers.

IDE. Insulin-degrading-enzyme plays a crucial role in the clearance of amyloid-β and has been proposed as a therapeutical target for Alzheimer's disease(Kurochkin et al., 2018).

ADAM10. ADAM10 is involved in the proteolytic processing of the amyloid precursor protein(Haass et al., 2012). ADAM10 also cleaves the ectodomain of the triggering receptor expressed on myeloid cells 2 (TREM2), to produce soluble TREM2 (sTREM2), which has been proposed as a CSF and sera biomarker of neurodegeneration(Yang et al., 2020).

NRGN. Neurogranin concentration in cerebrospinal fluid (CSF) is proposed as marker for synaptic dysfunction in age-related neurodegeneration(Casaletto et al., 2017), and has been shown to be specifically increased in patients with Alzheimer's disease(Willemse et al., 2018).

Synaptophysin. In the TMEV model, only a few large- to medium-sized synaptophysin/APP-positive bulbs were found in demyelinated areas. In MS patient tissue samples, the bulbs appeared exclusively at the inflammatory edges of lesions. In conclusion, our data suggest that synaptophysin as a reliable marker of axonal damage in the CNS in inflammatory/demyelinating conditions(Gudi et al., 2017).

PLCG1. Phospholipase C, gamma 1 gene mutations and abnormal splicing of PLCγ1 gene has been identified in AD using both high-throughput screening data and a deep learning-based prediction(Kim et al., 2021).

S6. Ribosomal protein S6

Phospho Tuberin T1462. The control of translation is disturbed in Alzheimer's disease (AD). Morel et al.(Morel et al., 2009) analyzed the crosslink between the up regulation of double-stranded RNA-dependent-protein kinase (PKR) and the down regulation of mammalian target of rapamycin (mTOR) signaling pathways via p53, the protein Regulated in the Development and DNA damage response 1 (Redd1) and the tuberous sclerosis complex (TSC2) factors in two beta-amyloid peptide (Abeta) neurotoxicity models. In SH-SY5Y cells, Abeta42 induced an increase of P(T451)-PKR and of the ratio p66/(p66+p53) in nuclei and a physical interaction between these proteins. Redd1 gene levels increased and P(T1462)-TSC2 decreased. These disturbances were earlier in rat primary neurons with nuclear co-localization of Redd1 and PKR. The PKR gene silencing in SH-SY5Y cells prevented these alterations. p53, Redd1 and TSC2 could represent the molecular links between PKR and mTOR in Abeta neurotoxicity. PKR could be a critical target in a therapeutic program of AD.

Tau, Phospho.Tau..S199. Phosphorylated tau.

References for eNote 1

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Supplemental Table 1 Antibody Information for Multispectral Immunohistochemistry

Target	Concentration	Antigen retrieval conditions	Vendor	Catalogue number	Host Species
Ab	1:500	EDTA ER2 pH9	Biolegend	803001	Mouse
Iba1	1:10,000	EDTA ER2 pH9	Wako	019-19741	Rabbit
GFAP	1:500	EDTA ER2 pH9	Dako/Agilent	Z033429-2	Rabbit
Cleaved Caspase 3	1:100	EDTA ER2 pH9	Cell Signaling	9664L	Rabbit
p-Tau	1:200	Citrate ER1 pH6	Invitrogen	MN1020	Mouse
DCX	1:5,000	Citrate ER1 pH6	Abcam	ab18723	Rabbit
CD68	1:500	Citrate ER1 pH6	Dako/Agilent	M0814	Mouse
PLP	1:400	Citrate AR buffer + TritonX100 pH6	PLP antibody source was[10]		Rat

Antigen retrieval was performed on a fully automated immunostainer using either ER1 (citrate with a pH range of 5.9-6.1) or ER2 (EDTA with a pH range of 8.9-9.1) buffers, except for PLP which was manually processed in the microwave with citrate AR buffer and TritonX100 (pH6).

Supplemental Table 2 Antibodies Used for nanoString

Item name	Item Number
GeoMx Human Protein Core for NGS	121300129
GeoMx Immune Cell Typing Assay	121300130
GeoMx Immune Activation Status Assay	121300132
GeoMx Myeloid Assay	121300137
GeoMx PI3K/AKT Signaling Assay	121300136
GeoMx Neural Cell Typing Assay	121300138
GeoMx Alzheimer's Pathology Assay	121300139
GeoMx Glial Cell Subtyping Assay	121300143

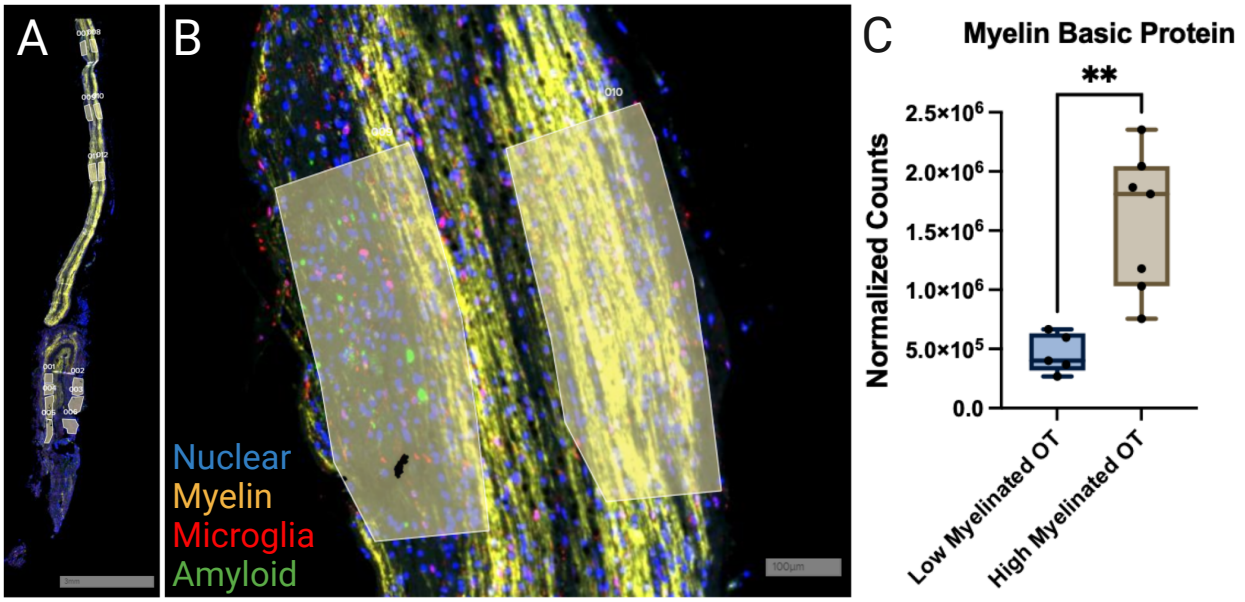
Supplemental Table 3 Antibody Information for Immunofluorescence for nanoString

Antibody ID	Name	company	clone #	Catalog #	concentration used
syto13	syto13	NanoString		121300306	25
MF-304	Beta amyloid	Novus	MOAB-2	NBP2-13075AF532	1:100
MF-618	MBP	Novus	2H9	NBP2-22121AF594	1:100
MF-643	Iba1	millipore	20A12.1	MABN92-AF647	1:100

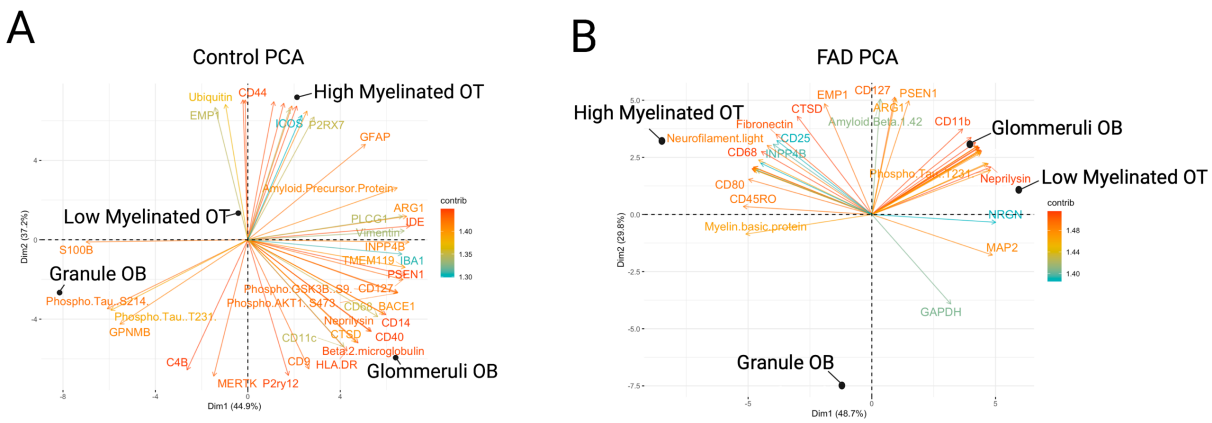
Supplemental Table 4. Excel worksheet with TempO-Seq raw counts and p-values for all genes for the olfactory bulb (control vs. FAD)

Supplemental Table 5. Excel worksheet with TempO-Seq raw counts and p-values for all genes for the olfactory tract (control vs. FAD)

Supplemental Table 6. Excel worksheet with genes included in specific pathways by Insight analysis.



Supplemental Figure 1. Quantification of expression of myelin basic protein in the high and low myelinated regions of the OT of FAD subjects using nanoString spatial profiling. (A) A representative horizontal tissue section (FAD 359) of the OB/OT showing regions used for protein quantification. The sections are immunolabeled for myelin basic protein, Iba1, Aβ and nuclear staining (styo13). (B) Closeup of OT areas with high and low myelination. (C) Normalized counts for myelin basic protein in the low and high myelination areas. The difference is statistically significant ($p<0.05$, pairwise t-test).



Supplemental Figure 2. Principal component analysis of the proteomic data for the control and FAD datasets.

A principal component analysis of nanoString protein expression shows increased distance between high and low myelinated OT in the FAD tissue. (A) Control. (B) FAD.