SUPPLEMENTARY MATERIAL FOR:

Vascular Pathology in Multiple Sclerosis: Reframing Pathogenesis Around the Blood-Brain Barrier

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Supplementary Table 1 - Potential associations of MS-associated genes with BBB function *(referenced version of Table 1 from Main Text).* The twenty highest ranking genes on DisGeNET were searched for in PubMed with the keywords 'blood brain barrier', 'endothelial', 'endothelium', 'astrocyte', 'pericyte' and 'tight junction'. Results were reviewed and the most relevant possible associations are discussed. *Data from UniProt (http://www.uniprot.org/)

Gene	Protein function*	Possible BBB associations
IL7R	Interleukin-7 cytokine receptor	Expressed by human microvascular ECs (although not specifically in brain). Exogenous IL-7 stimulates EC proliferation in a dose-dependent manner[1]. Expression of IL7R by non-bone marrow derived cells contributes to EAE pathogenesis in a mouse model. IL7R is expressed in mouse astrocytes[2].
IL2RA	Interleukin-2 cytokine receptor. A soluble form has also been isolated, thought to result from extracellular proteolysis.	IL-2 stimulates angiogenesis in human umbilical vein ECs, indicating that IL2RA is likely expressed[3]. Administration of recombinant IL-2 leads to BBB disruption. Intrathecal levels of soluble IL2R are higher in MS compared to controls, and correlated with BBB damage[4].
HLA-DRB1	HLA class II molecule	Two polymorphisms in ALCAM, a molecule involved in leukocyte migration across the BBB, modify *HLA-DRB1*1501* effect in MS, with a five-fold lower risk of MS in *HLA-DRB1*1501* individuals[5].
CLEC16A	Involved in regulation of mitophagy	Strongly induced in astrocytes in inflamed cerebral cortex[6].
CD58	Also known as LFA-3. Ligand of the CD2 glycoprotein found on T lymphocytes	Constitutively expressed by human brain microvascular ECs (HBMECs). Monoclonal antibodies to LFA-3 block proliferation of activated CD4 T cells that are incubated with HBMECs[7].
HLA-DRA	HLA class II molecule	None found.
TNFRSF1A	Receptor for TNF-α and lymphotoxin-α, forms DISC complex mediating apoptosis	TNFR1 knockout mice have reduced activation of astrocytes in response to LPS[8]. In humans, TNFR1 is expressed on vasculature of brain metastases and in non-specific brain inflammation, but minimally in normal controls, and was associated with increased BBB permeability on administration of TNF in mice[9]. Expression of TNFR1 found to be higher in brain microvessels than spinal cord microvessels in rats[10]. TNFR1 signalling implicated in the response to hypoxic-ischaemic injury and BBB breakdown in rats[11].
CD6	Adhesion molecule, involved in regulating T cell responses, acting as a costimulatory molecule and involved in the response to LPS	Expressed on lymphocytes. Interacts with ALCAM, which is expressed on resting human BBB ECs, to mediate migration of cells across BBB. CD6 blockade suppresses migration of CD4+ T cells[12]. CD6 on T cells required for efficient migration through BBB in EAE[13].
CBLB	Ubiquitin ligase that promotes substrate degradation by the proteasome. Negatively regulates TCR, BCR and FCER1 signalling pathways	Implicated in regulating LFA-1 activity. *In vitro*, Cbl-b deficient phagocytes displayed increased adhesion to endothelial cells[14]. Also identified as a potential caspase 8 substrate, in a study where caspase 8 inhibition abrogated the *ex vivo* formation of endothelial progenitor cells, suggesting a role of Cbl-b in neovascularisation[15].
KIF1B	Motor for anterograde transport of mitochondria	None found.
TNFSF14	Cytokine also known as LIGHT, that, through binding to LTBR, activates NFkB and stimulates proliferation of T cells.	Induces proinflammatory changes in ECs[16] through NFkB signalling[17] and restores vascular integrity in tumours by inducing pericyte contractility[18]. Platelet associated LIGHT mediates adhesion to endothelium[19].

APOE	Apolipoprotein found in chylomicron that binds lipoproteins and mediates their catabolism. Main cholesterol carrier in the brain.	Produced by astrocytes. ApoE deficiency has been shown to increase BBB permeability via an effect on MMP9 in EAE[20]. One isoform, APOE4, increases BBB susceptibility to injury by activating a cyclophilin A-MMP9 pathway in pericytes. In a mouse model, expression of APOE4 or deficiency of murine ApoE lead to neurodegeneration preceded by vascular effects[21]. ApoE has similarly been implicated in BBB integrity following experimental subarachnoid haemorrhage in mice[22,23]. Further, ApoE inhibits pericyte mobility, which is needed for cerebrovascular function, through a RhoA-mediated pathway[24]. However, in a study of dementia disorders, increased BBB permeability was not related to ApoE genotype[22] and a more recent GWAS study provided good evidence that the two SNPs in ApoE implicated in MS are in fact not associated with susceptibility to MS [25].
HLA-DQB1	HLA class II molecule	None found.
IFNB1	Cytokine, classically important in the innate immune response to viruses	Exogenous IFNß is an important treatment in RRMS. Shown to decrease BBB permeability in MRI studies[26] and *in vitro*[27]. Serum from patients treated with IFNß-1b causes reduced BBB permeability when applied *in vitro*[28].
IL1B	Cytokine in interleukin family. Classically described as a proinflammatory pyrogen	Increases BBB permeability by several mechanisms including: directly through PKCθ signalling in microvascular endothelium[29]; through production of an inflammatory response causing increased ICAM-1 and decreased TJ ZO-1 expression[30]; and through a chemokine reaction mediated by astrocytes[31]. Others report that IL-1b can cause neurodegeneration independently of a leaky BBB, using an adenovirus model in mice which disrupts the BBB but subsequently repairs[32]; chronic IL-1b exposure can cause BBB disruption without neurodegeneration[33].
HLA-DPB1	HLA class II molecule	None found
PTPRC	Phosphatase involved in TCR signaling	None found
EVI5	Regulator of cell cycle	None found
CIITA	Transcriptional coactivator of HLA class II gene	One study looked at CIITA expression in astrocytes and endothelial cells in response to cytokines. CIITA expression was increased in both astrocytes and endothelial cells by IFNγ, but this effect was inhibited by TNFα in ECs, leading the authors to conclude that ECs in the BBB are not inducible antigen presenting cells (APCs) but astrocytes are, and that ECs instead respond to T cell interactions by making the BBB more permeable[34]. Others speculate that since astrocytes in the white matter of MS subjects are deficient in ß2 adrenergic receptors that deficiency will reduce a suppressive action of CIITA via PKA, enabling astrocytes to function as facultative APCs. Fluoxetine, a PKA agonist, reduced development of focal inflammatory lesions[35].
ICAM1	Adhesion molecule found on ECs. Ligand for LFA1 integrin found on leukocytes; on binding enables transmigration of leukocytes into tissues.	Widely studied in terms of action in transmigration of inflammatory cells across the BBB. One recent study showed that patients with SPMS had autoantibodies against galectin 3, which increased ICAM1 levels and might contribute to BBB breakdown[36].

Supplementary Table 2 – Actions of disease modifying MS drugs at the BBB (*referenced version of Table 2 from Main Text)*. Drug names were searched for in PubMed with the keywords 'blood brain barrier', 'endothelial', 'endothelium', 'astrocyte', 'pericyte' and 'tight junction'. Results were reviewed and the most relevant associations are discussed. *Information on drugs derived from[37,38]

Drug	Drug action*	Effects at the BBB
IFNß-1a and -1b	Cytokines that modulate immune function	Reduces BBB permeability through actions on TJs and reduces expression of adhesion molecules for immune cell egress[39].
Glatiramer acetate	Pool of peptides antigenically similar to MBP with an immunomodulatory role	Specifically enhances Th2 lymphocyte migration across the BBB[40]. Enhances activated dendritic cells migration across the BBB, possibly through RhoA activation[41]. Enhances production of brain-derived neurotrophic factor (BDNF)[42] which decreases blood-spinal cord barrier permeability[43] - another related neurotrophic factor, GDNF, reduces BBB permeability[44].
Mitoxantrone	Immunosuppressive agent targeting DNA of immune cells	Globally cytotoxic[45], so likely does influence BBB but specific studies are sparse. Shown to influence PPARα in ECs in a BBB model[46]. Also has effects on microglia that could affect the BBB[47].
Dimethyl fumarate	Immunomodulatory agent, possibly through activation of Nrf2 pathway	Reduces cerebral oedema in a mouse stroke model[48]. Promotes BBB integrity through actions on TJs and decreased MMP activity through Nrf2 and casein kinase 2 signalling[49,50]
Natalizumab	Monoclonal antibody targeting alpha-4 integrin, affecting immune cell adherence to endothelium	Blockade of alpha-4 integrin (aka VLA-4) on lymphocytes and monocytes directly affects egress of cells through the BBB, and decreases the number of Gd-enhancing lesions on MRI[37,38]. One MRI showed that there was no difference between Natalizumab and placebo in levels of subtle BBB leakage in non-Gd enhancing lesions[51], but a recent *in vitro* study has shown that Natalizumab partially inhibits the BBB disruptive effect of soluble VCAM-1 on alpha-4 integrin expressed on ECs[52].

Fingolimod	Sphingosine 1-phosphate receptor inhibitor, affects lymphocyte egress from lymph nodes	In a study incubating sera from MS patients with brain microvascular ECs, fingolimod prevented BBB disruption by upregulating the TJ protein claudin-5 and downregulating the adhesion molecule VCAM1[53]. Similar roles in preserving BBB integrity have been shown in EAE models[54]. However, another study showed that Fingolimod did not enhance BBB integrity in inflammatory conditions and in fact was associated with decreased expression of occludin[55]. Others say Fingolimod reduces EC death in the presence of inflammatory cytokines[56]. Astrocyte production of ceramides, which disrupt BBB function, is inhibited by Fingolimod in MS. In particular, there are striking increases in ceramide production in astrocytic endfeet, suggesting a direct link with the BBB[57]. Importantly, S1P1 modulation on astrocytes is required for Fingolimod efficacy in EAE[58].
Teriflunomide	Dihydroorotate dehydrogenase inhibitor, affecting pyrimidine synthesis in immune cells	No known direct link
Alemtuzumab	Monoclonal antibody targeting CD52, causing depletion of monocytes and lymphocytes	One review suggests a potential link to 'recovery of BBB integrity' since anti-CD52 therapy restored intestinal epithelial function in a mouse model of inflammatory bowel disease [59]; otherwise no direct link known.
Daclizumab	Monoclonal antibody targeting CD25, a component of the IL2 receptor, reducing T cell responses	No direct link known - one paper suggests that since a reduction in contrast enhancing lesions in MRI takes ~2 months to occur, then Daclizumab doesn't have a BBB effect but works through immunomodulation[60].
Ocrelizumab	Monoclonal antibody targeting CD20 that depletes B cells	No known direct link
Laquinimod	Immunomodulatory agent, acting through the quinolone-3-carboxamide pathway	Decreases T cell migration into the CNS in an EAE model through an effect on the endothelium. *In vitro* treatment reduces expression of ICAM-1 and ALCAM on endothelium, increases expression of adherent junction protein p120 and TJ protein ZO-1, and increases transendothelial electrical resistance indicating a direct effect on BBB integrity. Also decreases Th1 and Th17 lymphocyte transmigration[61] and downregulates the astrocytic response[62]. One poster abstract suggests that laquinimod reduces MMP-7 and -10 and VEGF-A expression (all of which are implicated in BBB disruption) in response to IL-1b treatment in vivo[63].

Supplementary Table 3 – Additional References for the Main Text. The complete list of pertinent references for the information included in the main text are provided below.

Section title	**Additional references**
Introduction	[64–74]
BBB in health	[75–91]
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Synthesis and conclusion	[159,160]

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