

# Choroid plexus imaging to track neuroinflammation – a translational model for mouse and human studies

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The choroid plexus (ChP) is a highly vascularized and secretory tissue in each of the brain ventricles that represents the key structure between the blood and the cerebrospinal fluid (CSF). Besides its essential role in CSF production and brain waste clearance pathways, the ChP also contributes to the regulation of central nervous system (CNS) immunosurveillance (Ghersa-Egea et al., 2018). Indeed, the ChP forming the blood-CSF barrier (BCSFB) regulates the entry of immune cells and solute molecules into the brain and *vice versa*. When antigen-specific, autoreactive immune activation occurs in the periphery, inflammatory cells migrate through the brain barriers towards the CNS (Strominger et al., 2018), initiating neuroinflammatory diseases such as multiple sclerosis (MS).

A clear relationship of ChP integrity and molecular signatures of neuroinflammation was observed showing the involvement of up-regulated functional pathways involving T cell adhesion, differentiation and activation (Fleischer et al., 2021). In postmortem studies, the ChP of MS patients is characterized by more antigen-presenting cells in the stroma, T cell infiltration, disruption of tight junctions in the ChP epithelium and endothelial overexpression of lymphocyte adhesion molecules (Vercellino et al., 2008).

Recently, an imaging study suggested that the morphology of the ChP may serve as a surrogate marker for MS disease activity (Fleischer et al., 2021). A larger ChP volume in MS patients and in two mouse models mimicking MS constituted a magnetic resonance imaging (MRI)-detectable correlate of immune cell trafficking into the CNS rather than a mere readout of clinical performance (Fleischer et al., 2021). Here, the ChP volumes were not only significant determinants that influence Expanded Disability Status Scale development in MS over time, but also outperform conventional MRI biomarkers like T1 contrast-enhancing lesions and new T2 lesions (Fleischer et al., 2021). Hence, altered volume of the ChP provides a structural interspecies biomarker of inflammatory disease activity in the early phase of MS, whereas long-term dynamics throughout the disease course are unknown. An *in vivo* translocator protein positron emission tomography study further revealed not only an enlarged but also inflamed ChP in patients with MS (Ricigliano et al., 2021).

Despite the scarcity of evidence available from clinical, translational, and neuropathologic studies, these investigations highlight the impact of immune homeostasis within the ChP in shaping the brain's structural integrity and the clinical phenotype under neuroinflammation.

In humans, the ChP has been evaluated using

various imaging modalities such as structural T1-weighted MRIs (where ChP has intensity similar to grey matter voxels), arterial spin labeling, positron emission tomography, and diffusion-weighted imaging. However, the gold-standard technique to non-invasively segment ChP is to manually delimit it on T1-weighted MRIs enhanced with a gadolinium-based contrast agent. The fenestrated endothelium in ChP allows contrast to accumulate in the interstitium, while the BCSFB barrier precludes a contrast agent to leak into CSF (Shi et al., 2017). However, manual segmentation is time-consuming and impractical for large cohorts, where automated methods are preferred, and several such as FreeSurfer segmentation (Fischl, 2012) have already conventionally been used. Nevertheless, MRI intensity correction is required to assist in the localization of the region of interest and its anatomical boundaries, and the output of automated algorithms still requires further validation and improvement.

Regarding further MRI sequences, diffusion imaging metrics have been used in human ChPs with good feasibility to characterize the ChPs despite their small size. In one study, ChPs apparent diffusion coefficient, reflecting the molecular motion of water in the interstitial space, was shown to increase with increasing age (Alicioglu et al., 2017). This effect may be related to increased water diffusion across the epithelium via paracellular spaces, thus signaling BCSFB malfunction. Another study, using contrast-enhanced T2-weight perfusion imaging, reported that the mean permeability of the capillaries inside the ChP decreases with age, whereas the mean transit time (perfusion) increases (Bouzerar et al., 2013).

Integrating imaging metrics from MRI and molecular signatures using RNA sequencing is very important to replicate the findings in different datasets. We were able to replicate our previous findings (Fleischer et al., 2021) in an independent open dataset. The results are shown in **Figure 1** separately for MS patients, the experimental autoimmune encephalomyelitis (EAE) model, and for cuprizone mouse model.

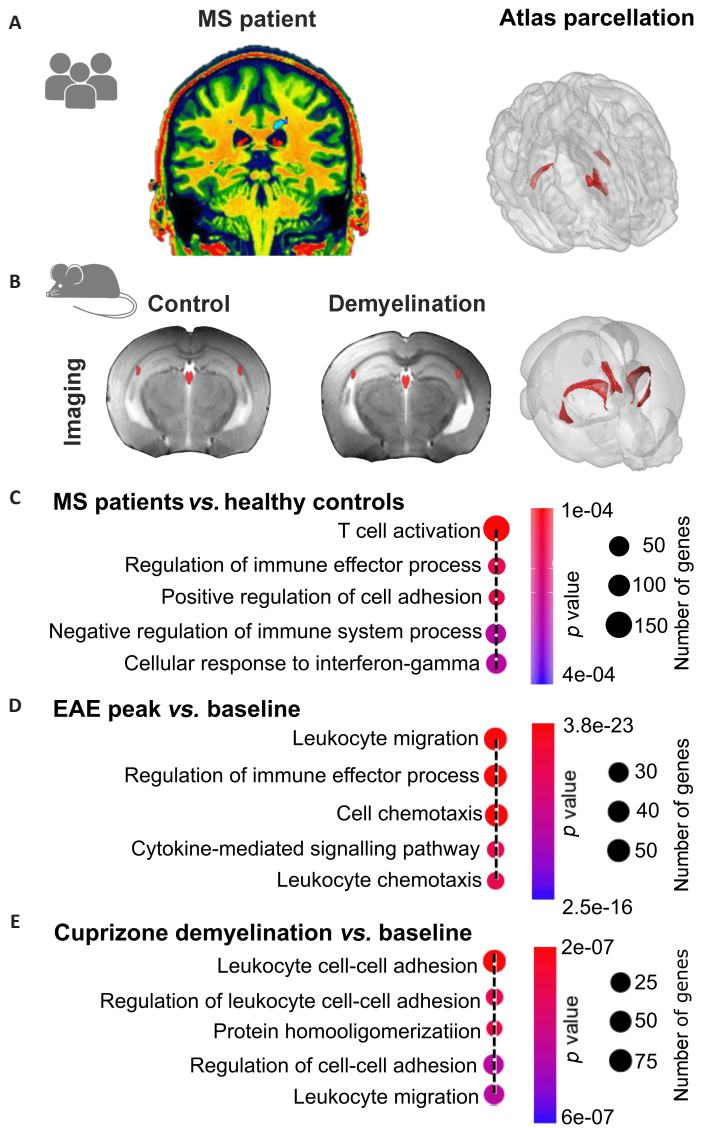
Under physiological conditions, the ChP maintains CNS immune homeostasis by providing local activation and subsequent migration of CNS-specific T cells across the choroidal epithelium into the CSF spaces. Two main mechanisms explaining the morphological and molecular transformations of the ChP upon neuroinflammation (patients with MS, mice with EAE) and exposure to toxic substances (cuprizone in a mouse model of MS) may be assumed.

First, ChP mediates the passage of adaptive

immune cells through the BCSFB, as increased numbers of CD3<sup>+</sup> T cells in the ChP stroma are observed in EAE mice compared to naïve mice and in cuprizone-diet mice compared to untreated mice (Fleischer et al., 2021). In MS patients, the ChP stroma harbor increased numbers of T cells and CD138<sup>+</sup> plasma cells, and show increased expression of vascular cellular adhesion molecule-1 (Vercellino et al., 2008), suggesting that inflammation of the ChP precedes the development of CNS demyelinating lesions. Obviously, the inflammatory cells and cytokines passed through the BCSFB together with the ChP secreted molecules alter the composition of the CSF. Studies of the CSF composition show increased numbers of activated T cells, B cells, and antibody-secreting cells, whose entry route into the CNS is the ChP (Kivisäkk et al., 2003; Kooij et al., 2014). EAE mice with deficient ChP tight junction proteins display enhanced inflammation in the ChP as determined by high numbers of CD45<sup>+</sup> leukocytes with the concomitant increase in a number of infiltrating leukocytes in their CSF (Kooij et al., 2014). Along with the migration of T cells from the periphery, infiltration, and activation of microglial cells (Iba1<sup>+</sup> and Clec7a<sup>+</sup>), within the ChP stroma of EAE and cuprizone-diet mice are also observed (Fleischer et al., 2021). Microglial cells play a crucial role in maintaining chronic neuroinflammation that translates into neurodegeneration (Groppa et al., 2021). Recently, a mechanism preventing the entrance of large immuno-inflammatory molecules through the ChP barrier mediated by the up-regulated wingless-type, catenin-beta 1 signaling pathway was described (Carloni et al., 2021).

The second mechanism relates to mitochondrial injury with subsequent oxidative injury and hypoxia, primarily occurring upon exposure to cuprizone, a mitochondrial toxic compound. Functional activity of the ChP, a high-energy demanding process, is provided by the presence of numerous mitochondria in the ChP epithelium. Mitochondrial injury followed by the damage of choroidal epithelial cells results in disruption of the ChP barrier function that facilitates the passage of blood plasma constituents and subsequent enlargement of the ChP stroma. These ChP tissue pathological processes are mirrored by increased expression of mitochondria-related genes (Fleischer et al., 2021). However, at the same time, the up-regulation of mitochondrial functional pathways may be related as well to increased energetic demands of the ChP cells to maintain increased secretory activity in conditions of neuroinflammation.

In summary, MRI in mouse models is opening a window for translational studies, allowing the combined utility of molecular invasive measurements with noninvasive imaging to understand the role of the ChP in neuroinflammatory and neurodegenerative processes. As described in this perspective article, several studies have shown that ChP links to both neuroinflammation and neurodegeneration. Refined imaging approaches and advanced sequencing techniques generate readouts that robustly mirror disease activity and progression in MS, thereby further developing the translational research in this field.



**Figure 1 | Choroid plexus imaging and RNA sequencing upon neuroinflammation.**

Representative (A) human and (B) animal magnetic resonance images (MRI) with choroid plexus marked in red in the atlas and multiple sclerosis (MS) lesions in blue. The replicated RNA sequencing results from open datasets separately for (C) MS patients vs. healthy controls and the two mouse models – (D) experimental autoimmune encephalomyelitis (EAE) peak vs. baseline and (E) cuprizone demyelination vs. baseline.

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