Supplementary Methods

Clinical chemistry and immunological assessments

Plasma and CSF from non-fasting patients and controls were stored at -80°C at Uppsala Biobank. Routine CSF analysis included IgG indices (ref < 0.63)., age-related CSF/plasma albumin (AQ; ref: age > 6 months to 45 years, < 6.8), oligoclonal bands (OCBs) in serum and CSF and white blood cell count (ref < 5 cells per 10^6/L) was performed by an accredited medical laboratory (Uppsala University Hospital). CSF NfL (References: Age < 30 years, < 380 ng/L; 30-39 years, < 560 ng/L;)and GFAp concentrations were measured with enzyme-linked immunoassays (ELISAs) as previously described [1, 2], at Sahlgrenska University Hospital (References: Age < 20 years, < 175; 20-59 years, < 750 ng/L). CSF t-Tau concentration was measured using Lumipulse technology in accordance with the kit insert from the manufacturer (References: Age < 18 years, < 250 ng/L; 18-44 years, < 300 ng/L)(Fujirebio, Ghent, Belgium).

Immunofluorescence was used to test for IgG antibodies against NMDAR, leucine-rich glioma-inactivated 1 (LGI1), contactin-associated protein-like 2 (CASPR2), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and gamma-aminobutyric acid (GABA) receptors on transfected cells (Euroimmune, Lübeck, Germany). If the immunofluorescence screening indicated the presence of antibodies against intracellular neuronal antigens, an immunoblot was performed to test for specific IgG antibodies against Hu, Ri, Yo, Ma2, amphiphysin and CV2.

Measurement of Inflammatory markers in CSF and blood

CD27 belongs to the tumor necrosis factor (TNF) receptor family and is an antigen expressed primarily on naïve peripheral blood T lymphocytes [3]. Two studies have concluded that levels of the soluble form, sCD27, in CSF discriminates between inflammatory and non-inflammatory neurological disorders [4, 5]. SCD27 was analyzed with the human CD27/TNFRSF7 DuoSet Elisa from R&D systems (DY382-05) (Clinical chemistry, Uppsala University Hospital).

To detect a range of innate and adaptive immunological responses, metabolic inflammation as well as early signs of vascular injury we chose markers that have been informative in our earlier work [6-8] and in data from pilot studies. The analysis was performed by an accredited laboratory (SciLife, Uppsala). Electro-chemiluminescent signals were detected corresponding to cytokine targets using a SECTOR Imager instrument (Meso Scale). The MesoScale analyzing kits we used were: Vascular Injury V-plex (K15198D), Cytokine V-plex (K151A0H), Pro-inflammatory V-plex (K15049D and Chemokine V-plex (K15047D), custom assay (U-plex; CCL5, TNFSF13B, BDNF, bNGF, IFNA2, Leptin and CXCL12).

Inflammatory markers were further visualized using R software environment for statistical computing and graphics. Measurements included are those of controls n=51 (one control was excluded due to development of iritis after sampling) for plasma and n=7 for CSF.

Indirect Immunohistochemistry (IHC)

Brains for IHC were collected from 4% paraformaldehyde perfused p56 mice and frozen by submerging brain covered in FSC22 Frozen Section Comp (R-3801480, Leica) in -80°C 2 methylbutane (R-101576431, Sigma Aldrich) using dry ice. The sections were cut to 16 µm slices using a cryostat before they were attached to superfrost plus adhesion slides (R-10149870, Thermo Scientific). The slides were left to heat up to room temperature (RT) for approximately 60 min and encircled with a DAKO-pen (R-S2002, DAKO). After rehydration of sections in 1x Phosphate Buffered Saline (R-P5493, Sigma Aldrich) (PBS) for 15 min, patient CSF was diluted 1:4 in primary antibody diluent (0.3% Triton X-100 (R-T8787, Sigma Aldrich) + 0.1% Sodium azide (R-S2002, Sigma Aldrich) (NaN_3) in PBS and incubated for 16 h overnight at 4°C. The slides were then washed separately in 1x Tris Buffered Saline (TBS tablets R-097500-100, Medicago) and 0.01% Tween-20 (R-9005-54, Sigma Aldrich) (TBST) for 15 min followed by another round for 10 min and the last round for 5 min.

After 3 washes in TBST, sections were incubated in 0.5% TSA Blocking Reagent (R-FP1012) and 1:5000 DAPI (R-D1306, Invitrogen) (TNB-DAPI) in Tris-HCL (R-15567-027, Gibco) for 30 min at RT whereafter the first round of the glycerol (R-G5516, Sigma Aldrich) 1:2 diluted species-specific HRP-conjugated secondary antibody (1^{st}: goat-α-human IgG Fc R-A18829, Invitrogen) was further diluted 1:250 in TNB-DAPI and incubated 30 min at RT. Following additional 3 TBST washes, the primary patient CSF was developed with tyramide signal amplification (TSA) following the manufacturer's instructions with a first-round solution of 1:150 (1^{st}: Cy3.5-R-FP1484, Perkin Elmer) in 1x Plus Amplification diluent (R-FP1498, Akoya Bioscience) for 15 min and was followed by 3 TBST washes.

Following TSA, sections were incubated in 0.1% NaN_3 (R-S2002, Sigma Aldrich) in 1x PBS for 30 min. Next, sections were re-incubated in a second round with 1:250 concentration of 1:2 diluted glycerol species-specific HRP-conjugated secondary antibody (2^{nd}: goat-α-human IgA R-PA1-74395, Invitrogen) in TNB-DAPI and incubated 30 min at RT. Following additional 3 TBST washes, the primary patient CSF was developed with the second round for TSA with 1:150 (2^{nd}: fluorescein isothiocyanate R-FP1168, Perkin Elmer) (FITC), in 1x Plus Amplification diluent for 15 min and was followed by 3 TBST washes.

The last round of staining was begun with another incubation of 0.1% NaN_3 in 1x PBS for 30 min. The sections were re-incubated in a third round with 1:250 concentration of 1:2 diluted glycerol species-specific HRP-conjugated secondary antibody (3^{rd}: goat-α-human IgM (heavy chain) R-A18841, Invitrogen) in TNB-DAPI and incubated 30 min at RT. Following additional 3 TBST washes, the primary patient CSF was developed with the third round of TSA with 1:150 (3^{rd}: Cy5-R-FP1171, Perkin Elmer), in 1x Plus for 15 min and was followed by 3 TBST washes.

Lastly, the slides were mounted with glass covers using polyvinyl alcohol (R-P8136, Sigma Aldrich) and DABCO (R-290734, Sigma Aldrich) (PVA-DABCO).

Microscopical assessments

The immunostained sections were scanned using the microscope Zeiss imager Z2 and Metafer slide scanning system. Images were specifically taken on Cortex, the CA1 region of hippocampus (subiculum included), the CA3 region of Hippocampus, Dentate gyrus, Thalamus, Cerebellum and of the background. Using ImageJ, the analysis of individual

sections begins with a mean intensity measurement of the background from specific TSA channels (Cy3.5, n=4, FITC, n=4 and Cy5, n=3). The selected 6 remaining regions were then assessed in relation to relevant subregions; lacunosum, oriens layer, pyramidal layer, radiatum layer, granule cell layer, molecular layer, polymorph layer, granular layer and purkinje layer. The mean, median, 95% CI and 75% CI of intensity values were measured and the background TSA specific channel measurement was subtracted to eliminate nonspecific binding.

The staining patterns have been examined microscopically by four observers (J.M., N.S., J.L.C. and I.L) and determined by morphological similarities to known cell markers and cell subunits.

Methodological error

The methods used in this study are limited in the following ways. The ability to adequately assess the type of cell and sublocation is limited without colocalization studies hence it is only possible to state a likeness to said structures. The evaluation of only three cases limits the statistical performance and implications, consequently, only raise interest in further and larger studies of similar entities.

Proteomics

Sample preparation and mass spectrometry, including data handling, followed the workflow by Sundblom et. al. (1)

Proteomics sample preparation

The low abundant proteins were enriched through the depletion of the most highly abundant proteins (albumin, IgG, alpha-1-antitrypsin, IgA, haptoglobin, transferrin, and fibrinogen) within the CSF and digested using a mixture of Trypsin and Lys-C. 260 µl CSF per sample was sterilely filtered using a cellulose acetate spin filter of pore size 0.22 µm (Agilent Technologies) at 15,000 rcf for 2 min before 250 µl was recovered for depletion via the human multiple affinity removal system (MARS) spin cartridge Hu-7 (Agilent Technologies, Palo Alto, CA). The flow-through (FT) collected via centrifugation for 2 min at 100 rcf was pooled with the first two wash (W) fractions eluted in 400 µl MARS-7 Buffer A each and subsequently dried in a SpeedVac (Thermo Fisher Scientific, Walham, MA). The MARS spin cartridge was operated according to the manufacturer's instruction. Throughout the depletion process samples were kept at 6 °C when allowed for by experimental design. Samples were redissolved in 50 µl denaturing buffer (6M urea, 50 mM NH_4CO_3). Following treatment with 10 µl 45 mM DTT at 50 °C for 150 min samples were re-equilibrated to RT. The DTT mediated reduction of disulphide bridges was maintained via cysteine methylation. To this end, 10 µl 100 mM IAA was added before a 15 min dark incubation. 50 µl 100 mM NH_4CO_3 and subsequently Trypsin/Lys-C dissolved in 500 mM NH_4CO_3 was added, resulting in a trypsin to target protein concentration of 5 % (w/w). Samples were digested O/N at 37 °C. A final purification and desalting step was performed with Pierce C18 Spin Columns (Thermo Fisher Scientific). The columns were activated by 50 % (v/v) ACN via two 200 µl pulses. Equilibration was similarly performed but with 0.5 % (v/v) aqueous TFA. 40 µl sample was loaded twice before columns were washed 3 times with 200 µl 0.5 % (v/v) TFA. The peptides were ultimately eluted in 3 times 50 µl 70 % (v/v) ACN and dried.

LC-MS/MS

Mass spectrometry was performed in a QExactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) using a nano electrospray ion source. A reversed phase liquid chromatography EASY-nLC 1000 system (Thermo Fisher Scientific) equipped with a 2 cm EASY-column (1D 100 µm, 5µm C18) as precolumn (Thermo Fischer Scientific) and a 10 cm EASY-column (1D 75 µm, 3 µm, C18) as analytical column (Thermo Fisher Scientific) was used for peptide separation. Elution was performed over a 150 min linear gradient 4-100 % (v/v) ACN at 250 nl/min. A survey mass spectrum of m/z = 400-1750, using the automatic gain control (AGC) target 3×10^6, with a resolving power of 70,000 at a full width half maximum bandwidth was acquired using positive ion mode. The top 10 intensity ranked ions were chosen for high-energy collision dissociation (HCD) fragmentation using 25 % normalized collision energy. Tandem MS spectra were generated with an AGC target of 5×10^5 at a resolution of 17,500. Since quantitation is only possible relative samples of the same run, and drift can produce false differences, the sample run order was planned to avoid this artifact.

Mass Spectrometry Data Handling

MaxQuant 1.5.1.2 was used for data processing before the Andromeda search engine could match fragmentation spectra with a FASTA database of human proteins extracted from the Uniprot database (release May 2020). In estimation of the identification false discovery rate (FDR), a decoy database including contaminants as well as a reverse database was used. An FDR of 1 % was accepted. The search parameters included: maximum 10 ppm and 0.6 Da error tolerances for the survey scan and MS/MS analysis, respectively. Additionally, only one missing trypsin cleavage site was allowed. Carbamidomethylation was set as static modification and oxidation (M) as variable. A protein match was not considered true unless two overlapping peptides were found. Label free quantification was used for comparative proteomics.

Pathway analysis

Pathway analysis was conducted in two separate pipelines. First, pathway over representation analysis (ORA) and gene set enrichment analysis (GSEA) was performed on differential expression (DE) data using a suite of Bioconductor R packages to query the WikiPathways database and generate network images of selected pathways within Cytoscape [9-14]. Log2FC, and p-values were calculated for gene products detected across all samples (three cases and three controls). The pathway ORA was conducted against the background of all significantly regulated proteins (p-value < 0.05) to pull pathways from the WikiPathways database passing a p-value cut-off of 0.05. After adjusting for false discovery rates the top pathways were imported to Cytoscape where the whole set of universally detected proteins was used to colour corresponding network nodes according to values of log2FC. The GSEA was similarly performed against the WikiPathways database using a list of proteins ranked according to log2FC. Finally, a composite picture of select WikiPathways was generated to highlight regulated regions of broader pathway annotations.

In a second independent pathway analysis, a t-test was run to compare differential protein expression between OCD and normal control CSF fluid. The top 40 differentially expressed proteins were illustrated in a heatmap and the top 20 of these were mapped onto pathways using multiple bioinformatics solutions: Qiagen IPA, G-profiler, String database and Oracle healthcare datamodel. P-values were adjusting for false discovery rates.

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