Anti-brain protein autoantibodies are detectable in extraparenchymal but not parenchymal neurocysticercosis

R. Michael E. Parkhouse, Arturo Carpio, Maria Milagros Cortez, Alex von Kriegsheim, Constantin Fesel

PII: S0165-5728(20)30026-6

DOI: https://doi.org/10.1016/j.jneuroim.2020.577234

Reference: JNI 577234

To appear in: *Journal of Neuroimmunology*

Received date: 14 January 2020

Revised date: 2 April 2020

Accepted date: 4 April 2020

Please cite this article as: R.M.E. Parkhouse, A. Carpio, M.M. Cortez, et al., Anti-brain protein autoantibodies are detectable in extraparenchymal but not parenchymal neurocysticercosis, *Journal of Neuroimmunology* (2019), https://doi.org/10.1016/j.jneuroim.2020.577234

Anti-brain protein autoantibodies are detectable in extraparenchymal but not parenchymal neurocysticercosis

R. Michael E. Parkhouse[1] Arturo Carpio[2,3] Maria Milagros Cortez[4], Alex von Kriegsheim[5], Constantin Fesel[1]

[1]Institute Gulbenkian de Ciencia, 6, Rua Quinta Grande, Oeiras 2780-156 Oeiras. Email: parkhous@igc.gulbenkian.pt.

[2] School of Medicine, University of Cuenca, Av. 12 de Abril y Av. Loja Cuenca, Ecuador. arturo.carpio@ucuenca.edu.ec

[3]G. H. Sergievsky Center, Columbia University, New York, New York

[4] Instituto de Investigaciones Biomédicas "Dr. Francisco J. Triana-Alonso". (BIOMED-UC) Facultad de Ciencias de la Salud, Sede Aragua, Universidad de Carabobo. Av. Las Delicias. Maracay. PO Box: 2351. Aragua. Venezuela. milacortez3@yahoo.com

[5] Edinburgh Cancer Research UK Centre Institute of Genetics and Molecular Medicine University of Edinburgh Crewe Road South Edinburgh EH4 2XR

Alex.VonKriegsheim@igmm.ed.ac.uk

cfesel@igc.gulbenkian.pt

Corresponding authors

R. Michael E. Parkhouse, Instituto Gulbenkian de Ciencia

6, Rua Quinta Grande, Oeiras 2780-156 Oeiras. parkhous@igc.gulbenkian.pt

Phone + 351 21 440 7939

Arturo Carpio

School of Medicine, University of Cuenca, Av. 12 de Abril y Av. Loja Cuenca.

Ecuador. arturo.carpio@ucuenca.edu.ec

Declarations of interest: None

Abstract

Neurocysticercosis (NC) presents a spectrum of clinical manifestations, with two broad clinical entities based on the central nervous system location of the parasite: extraparenchymal (EP-NC) and parenchymal (P-NC). In this work, using quantitative immunoblot methodology, we demonstrate the presence of autoantibodies to brain proteins in CSF from EP-NC, but not P-NC, patients. There was striking correlation between the level of autoantibodies and the levels of the secreted metacestode glycoprotein HP-10, suggesting that the level of stimulation of the autoantibody response may be a function of the number of **viable** parasites. Nine corresponding proteins autoantigens were provisionally identified by mass spectroscopy.

Keywords

Anti-brain autoantibodies, autoantigens, neurocysticercosis, extraparenchymal, parenchymal, HP-10.

Introduction

The presence of a variety of autoantibodies against brain tissues has been reported in patients with infectious diseases, autoimmune diseases, tumours and psychiatric disorders (Diamond et al, 2013; Margari et al, 2015; Uchibori and Chiba, 2015). Anti-brain antibodies have been reported in ~2 to 10% of healthy individuals (Hammer et al, 2014), but even those with pathogenic potential cause disease only if they traverse the blood brain barrier. Anti-brain autoantibodies that bind brain antigens can cause acute neuronal dysfunction if they penetrate the human central nervous system (CNS), and are increasingly recognized during infections, (Nestor et al, 2016; Mader et al, 2017). While their fine specificity and pathogenic roles in particular diseases remain unclear, such autoantibodies have immediate utility as diagnostic/prognostic "biomarkers", and may also provide a rational strategy for the development of novel treatments through the

elucidation of the differential molecular pathogeneses associated with these many diverse clinical manifestations.

Previous studies have suggested that parasites may trigger activation of both cellular and serological anti-brain autoimmune mechanisms (Rojas et al, 2018). Such autoantibodies have been explained by (1) Molecular mimicry between host and parasite molecules (Abu-Shakra et al, 1999; Nestor et al, 2016; Rojas et al, 2018) and (2) The release of immunogenic antigens normally "invisible" to the immune system upon parasite mediated cell lysis. The relative contribution of these two mechanisms to various autoimmune neuropathies requires further studies (Klein et al, 2017)

Neurocysticercosis (NC), infection of the human CNS by the larval phase of *Taenia solium*, is a neglected zoonotic (WHO, 2014) and usually poverty-related disease of high public health importance that is still a cause of unacceptable morbidity and mortality, not only in endemic lower-income Latin America countries, Africa, and Asia, but also increasingly in high-income countries due to migration (Symeonidou et al, 2018; O'Neal & Flecker, 2015). As the most common parasite of the CNS worldwide (Carpio et al, 2018), and a major cause of seizures and epilepsy in endemic countries (Tellez-Zenteno et al, 2017), NC is not a single entity. It is a "spectral" disease covering a wide range of clinical symptoms, ranging from benign to frankly life threatening, depending on the localization and physiological state of the parasite and the corresponding host inflammatory response. It is therefore surprising that the possible occurrence of anti-brain autoantibodies in the NC is unexplored.

Thus, the objective of this work has been to screen for the presence of autoantibodies to brain proteins in the cerebrospinal fluid (CSF) of clinically defined NC patients representative of the two main clinical forms of NC, extraparenchymal (EP-NC) and parenchymal (P-NC) invasion. Importantly, we detected such autoantibodies enriched in

cases of EP-NC over parenchymal P-NC, an observation that is consistent with our understanding of the immunological competence of these two compartments of the CNS.

2. Material and Methods

2.1 Study cases, Imaging examination and Samples.

Cerebrospinal fluid (CSF) samples were taken from 21 patients with NC and from 15 control neurological patients diagnosed as NC negative were collected from January 2015 to February 2016 by neurologists and neurosurgeons at five general hospitals in Cuenca, Ecuador, (Carpio et al, 2017; Parkhouse et al, 2017) **by lumbar puncture**. **Those patients with intracranial hypertension received mannitol (osmotic diuretic) 20%, 1 mg/kg, administered IV over 20 minutes, as a bolus (during and after the lumbar puncture procedure), in order to avoid complications due to raised intracranial pressure**. All participants suspected of NC underwent imaging studies [computed tomography (CT) and magnetic resonance imaging (MRI)]. The ethics committee at the University of Cuenca, Ecuador, approved this study, and signed, informed consent was obtained for all patients.

All of the positively diagnosed cases fulfilled the validated, imaging diagnostic criteria for NC. The cyst stage (vesicular, colloidal, granular-nodular and calcified) was based on interpretation of CT and MRI (Lerner et al, 2012). The final classification into the different diagnosis: definitive parenchymal NC, definitive extra-parenchymal NC, mixed parenchymal plus extraparenchymal NC and probable parenchymal NC) was according to the previous validated diagnostic criteria (Carpio et al, 2016). Neurosurgical patients who did not fulfil the diagnostic criteria for NC were selected as negative controls. Specifically, CSF samples were obtained from the following neurological patients: hydrocephalus (5), cerebral tumours (3), optical neuritis (2), head-

trauma (1), Gillian-Barre-syndrome (1), hygroma (1), meningeal cancer (1), Adenoma of the hypophysis (1)

2.2 HP10 antigen ELISA (HP10 Ag ELISA).

Both control and experimental CSF and serum samples were assayed in the HP10 Ag ELISA (Harrison et al, 1989; Fleury et al, 2013), which detects a secreted glycoprotein of viable metacestodes. Briefly, microtitre plates (Immulon 2HB) were sensitized overnight with the HP10 monoclonal antibody (10µg/mL) in carbonate/bicarbonate buffer, pH 9.6, and then blocked with bovine serum albumin (1% in PBS). After washing, duplicate samples of paired undiluted CSF or serum (50µL/well) were added. The bound HP10 Ag was then revealed with HP10-Biotin followed by Streptavidin Peroxidase and TMB substrate (Pierce). The optical density at 450 nm was recorded and the values presented are the mean of duplicate samples minus the blank, and with a variation of ≤ 10%. The mean and standard deviation for the negative control samples in the HP10 Ag ELISA were 0.015 ± 0.0106 and 0.027 ± 0.036 for CSF and serum samples, respectively, thus giving the very low cut-off values of 0.063 and 0.135 for the CSF and serum samples, respectively (calculated as Mean + 3SD), and thus permitting the identification of a low cysts burden. For deciding positivity in the ELISA, we have used an optical density of ≥0.2 as the operational cut off value for both CSF and serum samples.

2.3. Autoantibody measurement in CSF by quantitative immunoblot

Quantitative immunoblot was carried out as previously described (Fesel et al, 2012). Briefly, mechanically homogenized and solubilized human brain tissue (obtained from the Legal Medicine Service, Havana, Cuba, from a deceased person without preceding brain disease) was separated by standard discontinuous 10% SDS-polyacrylamide gel electrophoresis and electrotransferred onto two nitrocellulose membranes as described

(Haury M et al, 1994). The immunoblot membranes were then incubated with CSF samples diluted 1/2 in PBS/0.2% Tween-20 using a cassette system allowing the simultaneous testing of 28 samples in separate incubation slots per membrane (Immunetics, Cambridge, MA). Samples were incubated with CSF for 4h at room temperature, washed and then the entire membrane was incubated with the secondary alkaline phosphatase-coupled anti-human IgG (Sigma-Aldrich) for 90 min at room temperature. After development with the insoluble phosphatase substrate NBT/BCIP, membranes were densitometrically scanned. Subsequently, the membranes were stained for total protein with colloidal gold (Protogold, BritishBioCell, Cardiff, GB), then scanned in the spaces between incubation slots in order to adjust migration scales as described [Haury et al, 1994). The resulting standardized migration scale was divided into sections around the immunoreactivity bands, in which the respective areas under the densitometric profile above the baseline level were calculated for each section and divided by the section length. To normalize the resulting section wise average densities between the two membranes, they were further divided by the average standardized density of all sections measured for a general reactivity standard (a defined mixture of several EP/NCC samples, present in two replicates on each membrane. Standardized vectors representing all section reactivities of each sample were analysed by Principal Component Analysis (PCA) using the software IgorPro (WaveMetrics) as described (Fesel et al, 2005). Scores of the first principal component (covering 60% of the total data variance) were taken to represent each sample's standardized overall reactivity. PCA factor-1 scores optimize the one-dimensional representation of multivariate data and, in this case, since all factor loads for the individual sections were positive, constituted, and were interpreted as, a weighted measure of overall auto-reactivity in each sample.

2.4 Mass spectroscopy of proteins

Quantitative mass spectrometry profiling of antibody-antigen interactions was carried out using a previously described method to identify protein complexes by affinity purification. In brief, samples for mass spectroscopy were prepared by coprecipitation of complexes formed between antibodies in human cerebrospinal fluid (CSF) and proteins present in a lysate of human brain (purchased from Novus Biologicals Europe, Cat No NB820-59177). Aliquots of the brain lysate were mixed with cerebrospinal fluid (3h, Oº), and the immune complexes were selected by absorption to Dynabeads^{TM} protein G (Thermofisher Scientific Cat. No. 10003D). After mixing the tubes were rotated (3h, Oº), and the beads were washed x4 with cell lysis buffer (Cell signalling Technology, Cat. No. #9803). The beads were then transferred to clean eppendorff tubes, washed x 2 with 50mM tris -0.13M NaCl, pH 7.8) and stored at -70º until analysis. For the analysis, 5 samples of CSF from clinically characterised cases of EP-NC were individually processed. The negative controls: were: (1) The beads without CSF, but with brain lysate and (2) The beads with a sample of CSF from a clinically characterised case of P-NC and the brain lysate. Proteins bound to the antibodies were eluted by incubating the beads with 0.5 µg of modified Trypsin (Promega) 100 µl 2 M Urea, 50 mM Tris pH8, 1 mM DTT at 27°C for 30 minutes under vigorous shaking. The beads were subsequently removed and the digest was completed at 37°C overnight. Cysteines were blocked by adding Iodacetamide 10 mg/mL for 30 minutes in the dark. Samples were acidified by adding 1% TriFluor Acetic Acid (TFA) and desalted using stage-tips. Eluates were lyophilised and re-suspended in 12 µl 0.1% TFA/water. Aliquots of 5 µl were analysed using an LC-MS/MS systems comprising of an RSL-nano (Thermo Fisher) with 25 cm 1.6µm Aurora column (Ionoptiks) coupled to a Fusion Lumos mass spectrometer (Thermo Fisher). Peptides were separated using a 40 minute gradient, the mass

spectrometer operated at a 1 s cycle time with 120k resolution (MS), HCD fragmentation and rapid-scanning in the ion-trap (MS/MS). The data was searched against the human Uniprot database using the MaxQuant/Andromeda software suite.

2.5 Statistics

Group comparisons were made with the Mann-Whitney nonparametric test and correlation was analysed by Pearson's R and linear regression analysis, all using the software IgorPro (WaveMetrics). P-Values below 0.05 were considered significant.

3. Results

3.1 Clinical characteristic of the patients

The median age of participants was 49 years and slightly more were male (Table 1). According to the previous validated criteria (Carpio et al, 2016), 8 patients (38%) had definite P-NC and 13 (62%) had definite P-NC plus EP-NC (eight patients had both P-NC and EP-NC). Two patients of the P-NC group had just one cysts/granuloma. Among participants with EP-NC, 40.9% had subarachnoid cysts, 54% had intraventricular, and 15.5% had both subarachnoid and intraventricular cysts. Numbers respective to cyst stages (vesicular, colloidal, granular-nodular and calcified) are shown in Table 1. There were differences related to inflammatory characteristics between P-NC and EP-NC: 61% of EP-NC patients had high levels of proteins in the CSF, versus 25% of P-NC. Similarly, 84% of EP-NC patients had increased leukocytes versus 25% of P-NC patients. However, these differences were not statistically significant.

3.2 Demonstration of autoantibodies by quantitative immunoblot

Proteins from human brain tissue without preceding brain disease were separated by standard 10% SDS-polyacrylamide gel electrophoresis, and determination of the levels of anti-human brain protein autoantibodies in CSF samples from EP and P NC patients,

were determined by the scanning of quantitatively standardized western blots. Binding profiles of three representative samples of each group are shown in Figure 1. Two conclusions are immediately clear. First, anti-brain protein antibodies were detected in the EP-NC samples, whilst being virtually absent in the P-NC samples. Although individual binding profiles of EP-NC patients were qualitatively heterogeneous, this observation applied to both band-specific and overall anti-brain autoreactivity. The difference between both groups was highly significant (P=7E-5) when quantitatively analyzed for the most prominent single reactivity band (section 21, indicated in Figure 1 and analyzed in Figure 2A), as well as for overall anti-brain IgG reactivity, represented by the first principal component score derived from all reactivity bands (P=2E-5, Figure 2B), or cumulative reactivity to all western blot bands (P=4E-5, not shown).

Second, we studied whether there was a positive correlation between the level of autoantibodies and the level of the HP10 glycoprotein in the CSF of the EP-NC patients. As the HP10 glycoprotein is secreted by viable metacestodes, then its level in CSF reflects the number of parasites. Thus, the level of stimulation of the auto-antibody response may be a function of the number of parasites, with high HP10 levels correlating with high levels of anti-brain protein autoantibodies. Indeed, we found a striking correlation of both (Figure 3A). Furthermore, the absence of anti-brain protein autoantibodies in cases of P-NC patient was particularly significant as such antibodies were clearly present in a variety of other cerebral pathologies (Figure 3B).

3.3 Identification of autoantigens by mass spectroscopy.

To identify the brain proteins recognised by the autoantibodies, CSF samples from EP-NC and P-NC patients were immobilised onto magnetic Protein-G beads. The loaded beads and the two negative controls (Protein-G beads alone or with CSF from a P-NC patient) were incubated with human brain lysate and, proteins bound to the beads were

identified and quantified by label-free, quantitative mass spectrometry. To determine proteins enriched by the autoantibodies, we generated a ratio of the intensity over the negative control (Protein -G beads alone) and used a harsh 16fold cut-off. Of 118 identified proteins, 46 fulfilled these criteria. Unsurprisingly, the most abundant proteins detected as enriched were immunoglobulins. After excluding these, 21 proteins remained (Table 2). Some of these, such as Albumin, Complement C4-B, FCGBP and FCGR1A, broadly bind immunoglobulins, whereas others have no such reported function and may be considered as possible specific antigens. Gene-ontology enrichment revealed that the majority of the proteins are reported to be secreted and/or involved in the immune effector process or defence response. **To further determine the specificity of immunoglobulins isolated from EP-NC CSF**, we used as the negative control beads incubated with CSF from a P-NC patient and similarly compared the intensities between the EP-NC and P-NC groups. Using a 1.5 fold enrichment cut-off, we determined that 14 of the same proteins were indeed enriched by extraparenchymal EP-NC over parenchymal P-NC immunoglobulins (Table 2). After excluding the immunoglobulins and immunoglobulins binding proteins, the remaining proteins may therefore be provisionally considered as potential targets of an EP-NC related autoimmune response. Specifically, the nine proteins were: Clusterin (CLU), Transthyretin (TTR), Haptoglobin (HP), Ceruloplasmin (CP), Alpha-2-Macroglobulin (A2M), CARD9 (CARD9), Cytostatin C (CST3), Angiotensin (AGT), 60S ribosomal protein L27a (RP27A), all of which were detected in at least 3 of the 5 extraparenchymal samples, graphically presented in the "Boxplot" (Fig. 4).

4. Discussion

Neurocysticercosis (NC) presents a spectrum of clinical symptoms, with two broad clinical entities based on the CNS location of the parasite; parenchymal (P-NC) and

extraparenchymal (EP-NC). In this work, using quantitative immunoblot methodology, we demonstrate **for the first time** the presence of autoantibodies to brain proteins in CSF from EP-NC. Levels of autoantibodies in the CSF of P-NC patients were either undetectable or considerably reduced. There was striking correlation between the level of autoantibodies and the levels of the secreted metacestode glycoprotein HP-10, suggesting that the level of stimulation of the autoantibody response may be a function of the number of **viable** parasites. Examination of the immunoblot profiles of the EP-NC samples revealed considerable heterogeneity between them. However, a total of nine proteins were identified by mass spectroscopy in at least 3/5 CSF samples from cases of EP-NC and thus may be provisionally considered to be possible common auto-antigens and worthy of further investigation. Gene-ontology enrichment revealed that the majority of these proteins are reported to be secreted and/or involved in the immune effector process or the defence response. The nine proteins were: Clusterin (CLU), Transthyretin (TTR), Haptoglobin (HP), Ceruloplasmin (CP), Alpha-2-Macroglobulin (A2M), CARD9 (CARD9), Cystatin C (CST3), Angiotensin (AGT), 60S ribosomal protein L27a (RP27A). Future work will focus on similar analysis of autoantibodies from further cases of EP-NC, and the potential role and application of theses autoantibodies to the diagnosis, prognosis and pathogenesis of the disease.

The observation of anti-brain protein autoantibodies in the CSF of EP-NC, but not P-NC is an important contribution towards understanding the immune response of humans with NC and one that is relevant to the known clinical and immunological differences between these two CNS compartments. The recent discovery that the CNS undergoes a constant immune surveillance within the meningeal compartment, through functional lymphatic vessels lining the dural sinuses (Louveau et al., 2015), is consistent with our

observations of considerably higher level of autoantibodies in the CSF of EP-NC patients than in the CSF of P-NC patients.

Neurocysticercosis presents a complex spectrum of clinical manifestations, often related to the localization and physiological state (i.e. viable, degenerative and calcified) of the parasite and the corresponding host immune response (Carpio et al., 2018; Fleury et al, 2010). For example, it has been documented that parenchymal and extraparenchymal NC are clearly distinct entities from the pathophysiological, clinical and immunological points of view (Carpio et al, 2016). The parenchymal location comprises the brain and spinal cord tissues, and the extraparenchymal location includes the intraventricular and subarachnoid spaces in between the leptomeninges, arachnoids, and pia mater where the CSF is circulating. The evolution of the *T. solium* metacestode located in the parenchyma has been well documented and classified by anatomo-pathological and imaging studies as vesicular, colloidal, granular-nodular, and calcified phases of the parasite (Escobar et al, 1972). Such a well-defined evolution of the parasite has not been observed in the extraparenchymal location, especially in the basal cisterns where the parasite assumes the racemose form and emerges as a "cluster of grapes" without scolex (Escobar et al, 1972). In addition to these morphological differences, the clinical manifestations of P-NC are quite different from those of EP-NC (Carpio et al., 2018). Thus, when the parasites are localized in the parenchyma, the main clinical manifestations are seizures or focal neurological deficits, which are usually transient and with good prognosis. EP- NC, on the other hand, has different clinical manifestations, including intracranial hypertension, cranial nerve abnormalities, and hydrocephalus, which may develop into more severe clinical sequelae, including mortality (Fleury et al, 2011). Moreover, the nature of the immune surveillance in the parenchymal *vs* extraparenchymal areas (Engelhardt et al, 2017) provides an additional and crucial

consideration. Specifically, extraparenchymal cysts are surrounded by CSF, which interacts with the peripheral immune system through its afferent and efferent connections (Louveau et al 2015), whereas the parenchyma is without contact to the immune system. Thus the observation of autoantibodies in the CSF of EP-NC patients is consistent with the immunobiology of the extraparenchymal compartment.

The immunological-related inflammatory response plays a critical role in the pathogenesis of NC. An imbalance of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), might explain the inflammatory host response observed in NC patients (Aguilar-Rebolledo et al, 2001). Consistent with this hypothesis, a significant improvement was seen in patients suffering from subarachnoid NC and treated with the TNF α inhibitor etanercept (Nash et al, 2019). In spite of the fact that these preliminary data are anecdotal with no comparison group, they do show an association between the presence of TNF-α in CSF and active, symptomatic and complicated forms of NC. Previous studies have shown that high levels of TNF-α are present in CSF from patients with active subarachnoid NC, perhaps due to a higher influx of cells into the subarachnoid region relative to the parenchyma (Aguilar-Rebolledo et al, 2001).

The presence of locally produced oligoclonal IgG bands in CSF is long known to be associated with infections and autoimmune responses of the CNS. For example, neuroborreliosis-associated oligoclonal CSF antibodies reactive against both the pathogen *B. burgdorferi* and CNS self-antigens have been described. Interestingly, the anti-Borrelia and anti-self antibodies are not necessarily crossreactive, suggesting that the antimicrobial immune responses and/or neuroinflammation may involve, or secondarily induce, autoimmune sensitization (Martin et al, 1988; Kuenzle et al, 2007). Consistent with this, in subacute sclerosing panencephalitis some, but not all, CSF

oligoclonal bands, were reactive with the infectious agent, measles virus (Burgoon et al, 2005). Moreover, oligoclonal CSF IgGs serve as an established biomarker and diagnostic tool in the classic autoimmune disease multiple sclerosis. A recent study in multiple sclerosis patients found the targets of these antibodies to be ubiquitous intracellular proteins rather than brain-specific self antigens, suggesting a nonspecific secondary response, for example to damaged/dead cells, rather than a direct pathogenic involvement (Brändle et al, 2016). In contrast, other authors suggest direct pathogenic effects of the CSF anti-brain antibodies that occur in other infectious and non-infectious diseases of the brain. For example, autoantibodies to CNS neuronal surface antigens have been described in association with autoimmune encephalopathies which notably feature psychiatric in addition to neurological symptoms (Armangue et al, 2014, Pollak et al, 2016). Similarly, in systemic lupus erythematosus, antibodies to double stranded DNA that cross react with the neuronal N-methyl – aspartate receptor, have recently been linked to neurocognitive dysfunction (Mader et al., 2017; Arinuma, 2018). Infection of the CNS with herpes simplex virus can trigger anti-CNS autoimmunity associated with anti-GABA antibodies (Alexopoulos et al., 2018). Finally, a recent study suggests that NMDAR autoantibodies induced by persistent *Toxoplasma gondii* infection may be a novel pathological hallmark of chronic toxoplasmosis (Li et al 2018).

From the practical point of view, this work may also provide novel strategies for the management and therapy of NC; for example, autoantibodies in patients with NC might serve as a "biomarker" indicating the intensity of inflammation, which is a crucial factor to consider when attempting to reduce the morbidity of EP-NC during cysticidal treatment. Furthermore, this work raises two other rational clinical possibilities: immunosuppressive therapy and the blocking of potentially pathogenic autoantibodies

and other pro-inflammatory effects through the administration of normal immunoglobulin, as indeed have been reported in cases of lupus erythematosus (Yildirim-Toruner et al, 2011) and various neurological, in particular neuro-inflammatory, conditions (Uchibori and Chiba, 2015; Chen et al, 2019). **Further similar studies are necessary to explore practical possibilities.**

5. Conclusions

In conclusion, the finding of significant levels of autoantibodies in the CSF of EP-NC, but not P-NC, patients is consistent with our understanding of the immunological potential of these two distinct anatomical areas of the brain, and highlights the need for further studies explaining the relationships between autoimmunity, neuro-inflammation and NC. We hypothesize that neuro-inflammation during the degenerative or transitional phase, particularly in EP-NC, is a necessary prelude to the induction of anti-brain autoantibodies. Identification of the specific antigenic targets of these autoantibodies may provide clues as to their role in the pathogenesis of EP-NC, and thus new approaches for the diagnosis and treatment of disease.

Ethical Approval

This project was in accordance with the ethical standards of the Helsinki Declaration of World Medical Association (1964) as amended in 2008 and was approved by the ethics committee at the University of Cuenca, Ecuador. Signed, informed consent was obtained from all patients.

Acknowledgments

We would like to thank the 'Programa Prometeo' of the Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT) of Ecuador, for their support, to Dr. RME Parkhouse, and the following neurologists and neurosurgeons who provided the CSF and serum samples in their respective hospitals: Luis M. Piedra and Nelson

López, Hospital del Instituto Ecuatoriano de Seguridad Social; Mónica Pacurucu and Jenner Aguilar, Hospital Vicente Corral Moscoso; Luis C. Vintimilla and Ana M. Toral, Clínica Santa Ana; Pablo Peña-Tapia, Hospital Universitario del Río; Sebastián López, Hospital Santa Inés, in Cuenca, Ecuador. Mass spectrometry was supported by Wellcome Trust (Multiuser Equipment Grant, 208402/Z/17/Z).

References

1. Abu-Shakra M, Buskila D, Shoenfeld Y. 1999 Molecular mimicry between host and pathogen: examples from parasites and implication. Immunol Lett. 67:147-52. DOI: 10.1016/s0165-2478(99)00004-8
2. Aguilar-Rebolledo F, Cedillo-Rivera R, Llagano-Violante P, Torres-López J, Muñoz-Hernandez O, Enciso-Moreno JA. 2001. Interleukin levels in cerebrospinal fluid from children with neurocysticercosis. J Trop Med Hyg. 64:35-40. DOI 10.4269/ajtmh.2001.64.35
3. Alexopoulos H, Akrivou S, Mastroyanni S, Antonopoulou M, Dinopoulos A, Giorgi M, Konstantinou K, Kouremenos E, Lariou M, Naoumis D, Pavlidou E, Pavlou E, Voudris K, Vlachoyiannopoulos P, Dalakas MC. 2018 Post-herpes simplex encephalitis: a case series of viral-triggered autoimmunity, synaptic autoantibodies and response to therapy. Ther Adv Neurol Disord. 11:1756286418768778. DOI 10.1177/1756286418768778.
4. Arinuma Y. 2018. Antibodies and the brain: anti-N-methyl-D-aspartate receptor antibody and the clinical effects in patients with systemic lupus erythematosus. Curr Opin Neurol 31:294-299. DOI 10.1097/WCO.0000000000000554
5. Armangue T, Leypoldt F, Dalmau J. 2014 Auto-immune encephalitis as differential diagnosis of infectious encephalitis. Curr Opin Neurol. 27:361–368. DOI 10.1097/WCO.0000000000000087

6. Burgoon MP, Keays KM, Owens GP, Ritchie AM, Rai PR, Cool CD, Gilden DH. 2005. Laser-capture microdissection of plasma cells from subacute sclerosing panencephalitis brain reveals intrathecal disease-relevant antibodies. Proc Natl Acad Sci U S A. 102:7245-50. DOI 10.1073/pnas.0502323102

7. Brändle SM, Obermeier B, Senel M, Bruder J, Mentele R, Khademi M, Olsson T, Tumani H, Kristoferitsch W, Lottspeich F, Wekerle H, Hohlfeld R, Dornmair K9 2016 Distinct oligoclonal band antibodies in multiple sclerosis recognize ubiquitous self-proteins. Proc Natl Acad Sci USA 113:7864-9. DOI 10.1073/pnas.1522730113.

8. Carpio A, Fleury A, Romo ML, Abraham R. 2018 Neurocysticercosis: the good, the bad, and the missing. Expert Rev Neurother. 18:289-301. DOI 10.1080/14737175.2018.1451328

9. Carpio A, Campoverde A, Romo ML, García L, Piedra LM, Pacurucu M, López N, Aguilar J, López S, Vintimilla LC, Toral AM, Peña-Tapia P. 2017. Validity of a PCR assay in CSF for the diagnosis of neurocysticercosis. Neurol Neuroimmunol Neuroinflamm. 4:e324. DOI 10.1212/NXI.0000000000000324.

10. Carpio A, Fleury A, Romo ML, Abraham R, Fandiño J, Durán JC, Cárdenas G, Moncayo J, Leite Rodrigues C, San-Juan D, Serrano-Dueñas M, Takayanagui O, Sander JW. 2016 New diagnostic criteria for neurocysticercosis: Reliability and validity. Ann Neurol. 80:434-42. DOI 10.1002/ana.24732

11. Chen Y, Wang C, Xu F, Ming F, Zhang H. 2019. Efficacy and Tolerability of Intravenous Immunoglobulin and Subcutaneous Immunoglobulin in Neurologic Diseases. Clin Ther. 41:2112-2136. DOI 10.1016/j.clinthera.2019.07.009.

12. Diamond B, Honig G, Mader S, Brimberg L, Volpe BT. 2013. Brain-reactive antibodies and disease. Annu Rev Immunol 31:345-385. DOI 10.1146/annurev-immunol-020711-075041.

13. Engelhardt B, Vajkoczy P, Weller RO. 2017. The movers and shapers in immune privilege of the CNS. Nat Immunol. 18:123-131. DOI 10.1038/ni.3666

14. Escobar A, Nieto D. Cysticercosis. 1972. In: Minkler J, ed. The Pathology of the Nervous System. New York: McGraw-Hill: 2507–2515.

15. Fesel C, Barreto M, Ferreira RC, Costa N, Venda LL, Pereira C, Carvalho C, Morães-Fontes MF, Ferreira CM, Vasconcelos C, Viana JF, Santos E, Martins B, Demengeot J, Vicente AM. 2012 Compensatory T-cell regulation in unaffected relatives of SLE patients, and opposite IL-2/CD25-mediated effects suggested by coreferentiality modeling PLoS ONE 7:e33992. DOI 10.1371/journal.pone.0033992

16. Fesel C, Goulart LF, Silva Neto A, Coelho A, Fontes CJ, Braga EM, Vaz NM. 2005 Increased polyclonal immunoglobulin reactivity toward human and bacterial proteins is associated with clinical protection in human Plasmodium infection, Malar J 4:5. DOI 10.1186/1475-2875-4-5

17. Fleury A, García E, Hernández M, Carrillo R, Govezensky T, Fragoso G, Sciutto E, Harrison LJ, Parkhouse RM. 2013 Neurocysticercosis: HP10 antigen detection is useful for the follow-up of the severe patients. PLoS Negl Trop Dis.7:e2096. DOI 10.1371/journal.pntd.0002096.

18. Fleury A, Carrillo-Mezo R, Flisser A, Sciutto E, Corona T. 2011. Subarachnoid basal neurocysticercosis: a focus on the most severe form of the disease. Expert Rev Anti Infect Ther. 9:123-33. DOI 10.1586/eri.10.150.

19. Fleury A, Escobar A, Fragoso G, Sciutto E, Larralde C. 2010. Clinical heterogeneity of human neurocysticercosis results from complex interactions among parasite, host and environmental factors. Trans R Soc Trop Med Hyg. 104:243-50. DOI 10.1016/j.trstmh.2010.01.005.

20. Hammer C, Stepniak B, Schneider A, Papiol S, Tantra M, Begemann M, Sirén AL, Pardo LA, Sperling S, Mohd Jofrry S, Gurvich A, Jensen N, Ostmeier K, Lühder F, Probst C, Martens H, Gillis M, Saher G, Assogna F, Spalletta G, et al. 2014. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. Mol Psychiatry 19:1143-1149. DOI 10.1038/mp.2013.110.

21. Harrison LJ, Joshua GW, Wright SH, Parkhouse RM. 1989 Specific detection of circulating surface/secreted glycoproteins of viable cysticerci in *Taenia saginata* cysticercosis. Parasite Immunol. 11:351-70.

22. Haury M, Grandien A, Sundblad A, Coutinho A, Nobrega A. 1994. Global Analysis of Antibody Repertoires. 1. An Immunoblot Method For the Quantitative Screening of a Large Number of Reactivities. Scand J Immunol 39: 79-87

23. Klein RS, Garber C, Howard N. 2017 Infectious immunity in the central nervous system and brain function. Nat Immunol. 18:132-141. DOI 10.1038/ni.3656.

24. Kuenzle S, von Büdingen HC, Meier M, Harrer MD, Urich E, Becher B, Goebels N. 2007 Pathogen specificity and autoimmunity are distinct features of antigen-driven immune responses in neuroborreliosis Infect Immun 75:3842-7. DOI 10.1128/IAI.00260-07

25. Lerner A, Shiroishi MS, Zee CS, Law M, Go JL. 2012 Imaging of neurocysticercosis Neuroimaging Clin N Am. 22:659-76. DOI 10.1016/j.nic.2012.05.004

26. Li Y, Viscidi RP, Kannan G, McFarland R, Pletnikov MV, Severance EG, Yolken RH, Xiao J. 2018 Chronic *Toxoplasma gondii* Infection Induces Anti-N-Methyl-d-Aspartate Receptor Autoantibodies and Associated Behavioral Changes and Neuropathology. Infect Immun. 86. pii: e00398-18. DOI 10.1128/IAI.00398-18.

27. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J. 2015 Structural and functional features of central nervous system lymphatic vessels. Nature. 523:337-41. DOI 10.1038/nature14432.

28. Mader S, Brimberg L, Diamond B. 2017 The role of brain-reactive autoantibodies in brain pathology and cognitive impairment. Front Immunol. 8:1101. DOI 10.3389/fimmu.2017.01101.

29. Margari F, Petruzzelli MG, Mianulli R, Lampa MG, Pastore A, Tampoia M. 2015 Circulating anti-brain autoantibodies in schizophrenia and mood disorders. Psychiatry Res. 230:704-8. DOI 10.1016/j.psychres.2015.10.029.

30. Martin R, Ortlauf J, Sticht Groh V, Bogdahn U, Goldmann SF, Mertens HG. 1988 *Borrelia burgdorferi*-specific and autoreactive T-cell lines from cerebrospinal fluid in Lyme radiculomyelitis. Ann Neurol. 24:509-16. DOI 10.1002/ana.410240406

31. Nash TE, Ware JM, Coyle CM, Mahanty S. 2019 Etanercept to control inflammation in the treatment of complicated neurocysticercosis. Am J Trop Med Hyg. 100:609-616. DOI 10.4269/ajtmh.18-0795.

32. Nestor J, Gata-Garcia A, Arinuma Y, Fujieda Y, Kowal C, Diamond B. 2016 Immune-mediated brain pathology: from autoantibodies to microglia. Discov Med. 22:201-207.

33. O'Neal SE, Flecker RH 2015. Hospitalization Frequency and Charges for Neurocysticercosis, United States, 2003–2012. Emerg Infect Dis 21: 969–76. DOI 10.3201/eid2106.141324.

34. Parkhouse RME, Carpio A, Campoverde A, Sastre P, Rojas G, Cortez MM. 2017. Reciprocal contribution of clinical studies and the HP10 antigen ELISA for the diagnosis of extraparenchymal neurocysticercosis. Acta Trop. 178:119-123. DOI 10.1016/j.actatropica.2017.11.005

35. Pollak TA, Beck K, Irani SR, Howes OD, David AS, McGuire PK. 2016 Autoantibodies to central nervous system neuronal surface antigens: psychiatric symptoms and psychopharmacological implications. Psychopharmacology (Berl). 233:1605-21. DOI 10.1007/s00213-015-4156-y.

36. Rojas M, Restrepo-Jiménez P, Monsalve DM, Pacheco Y, Acosta-Ampudia Y, Ramírez-Santana C, Leung PSC, Ansari AA, Gershwin ME, Anaya JM. 2018 Molecular mimicry and autoimmunity. J Autoimmun. 95:100-123. DOI 10.1016/j.jaut.2018.10.012

37. Symeonidou I, Arsenopoulos K, Tzilves D, Soba B, Gabriël S, Papadopoulos E. 2018. Human taeniasis cysticercosis: a potentially emerging parasitic disease in Europe. Ann Gastroenterol. 31:406-412. DOI 10.20524/aog.2018.0260

38. Tellez-Zenteno JF, Hernandez-Ronquillo L. 2017 Epidemiology of neurocysticercosis and epilepsy, is everything described? Epilepsy Behav. 76:146-150. DOI 10.1016/j.yebeh.2017.01.030.

39. Uchibori A, Chiba A. 2015 Autoantibodies in Guilliain-Barre Syndrome. Brain nerve. 67:1347-57 DOI 10.11477/mf1416200305.

40. WHO (World Health Organization). Assembling a framework for intensified control of taeniasis and neurocysticercosis caused by *Taenia solium*. Report of an

informal consultation. WHO Headquarters, Geneva, 17-8 July 2014. https://www.who.int/neglected_diseases/resources/9789241508452/en/

41. Yildirim-Toruner C, Diamond B. 2011. Current and novel therapeutics in the treatment of systemic lupus erythematosus. J Allergy Clin Immunol. 127:303-12. DOI 10.1016/j.jaci.2010.12.1087.

Fold enriched EP-NC/P-NC	Gene Name	Fold enriched EP-NC/Control	Gene Name
9.365095	C1QC	377.7808	C4B
4.93021	C1QB	249.8904	ALB
4.358646	CLU	188.9275	C1QC
4.319066	TTR	176.3019	TTR
3.219829	HP	160.7556	A2M
3.211777	C3	124.7022	CLU
2.507109	CP	107.6607	CP
2.1132	A2M	57.05606	FCGR1A;FCGR1B
2.036031	CARD9	54.58071	C1QB
2.031936	C4B	49.17738	APOA1
1.822976	CST3	45.19195	C3
1.778387	AGT	41.24662	CST3
1.722453	RPL27A	40.54411	HP
1.703668	ALB	32.91054	PTGDS
		31.11832	FGG
		29.15806	AGT
		27.52913	SERPINA1
		23.16631	B2M
		23.1236	FCGBP
		18.29663	CARD9
		16.14997	RPL27A

Table 2 Proteins selectively bound by antibodies in CSF from EP-NC

Protein G beads were incubated with CSF from cases of EP-NC and brain lysate. The bound proteins were analysed by mass spectroscopy filtering the results for the EP-NC with two negative controls. (1) Similarly processed beads, but including CSF from P-NC (EP-NC/P-NC in the Table head) and (2) Similarly processed beads, but lacking CSF (EP-NC/Control in the Table head). Details in Materials and Methods

Figure legends.

Figure 1 Western blot reactivity profiles of CSF IgG anti-brain autoantibodies. A) Blots developed with CSF from 3 cases of Extraparenchymal NC. B.) Blots developed with CSF from 3 cases of Parenchymal NC. C) Negative control without CSF but secondary antibody alone. D) Total protein staining. The most prominent reactivity band (section-21) is indicated in the Figure. Details in Materials and Methods.

Figure 2 Quantitative analysis of CSF anti-brain IgG autoreactivity in extraparenchymal and parenchymal NC. (A) Standardized optical densities of section-21 (as indicated in Fig. 1). (B) PCA factor-1 scores derived from the entire profiles, representing overall autoreactivity since factor loads were positive throughout. Black circles indicate CSF from cases of extraparenchymal NC. Black triangles indicate CSF from cases of parenchymal NC. Medians are shown for each group. Statistical significance for differences between both groups was calculated by the Mann-Whitney test.

Figure 3: **CSF HP10 correlates with IgG anti-brain**. (A) Linear correlation between levels of CSF HP10 (ELISA) and IgG anti-brain autoantibodies (PCA factor-1 scores) in CSF samples. Black circles indicate extraparenchymal CSF, open triangles indicate parenchymal NC CSF samples. The linear correlation coefficient R and the corresponding P-value resulting from linear regression analysis are indicated in the insert. (B) PCA factor-1 scores from CSF samples of patients with other cerebral pathologies. The horizontal bar represents their median and, reading from top to bottom, are: Normotensive hydrocephalus, Adenoma of the hypophysis, Guillain-Barré syndrome, Hygroma, Idiopathic hydrocephalus, Communicating hydrocephalus, head-

trauma, Normotensive hydrocephalus, Meningeal cancer, Optical neuritis, Tumour, Optical neuritis, Hydrocephalus, Tumour, Tumour.

Figure 4 "Boxplot" demonstrating protein autoantigens enriched by immunoprecipitation with antibodies in the CSF of EP-NC patients. The protein targets recognized by autoantibodies present in the CSF of five clinically defined cases of EP-NC and two clinically defined cases of P-NC were isolated by immunoprecipitation from a brain lysate using protein-G and submitted to analysis by mass spectroscopy. Using a 1.5 fold enriched cut-off, and filtering the results for the EP-NC samples with those of the P-NC samples, nine proteins were enriched in at least 3/5 of the EP-NC CSF samples. Details in Materials and Methods.

Table 1. Clinical characteristic of the patients

	Total	Cases	Controls*	P-value for cases vs. controls	P-NC **	EP-NC **
	N=36	n=21	n=15		n=8	n=13
Age in years, mean (SD)						
	49.0 (19.8)	35.9 (17.0)	58.4 (20.3)	P=.01	37.6 (15.8)	45.2 (17.4)
Sex, n (%)						
Male	21 (58.3%)	13 (61.9)	9 (60%)	P=.26	4 (50.0%)	9 (69.2%)
Female	15 (41.6%)	8 (38.1%)	6 (40%)		4 (50.0%)	4 (30.7%)
Symptoms						
Headache	—	18 (85.7%)	—	—	6 (75.0%)	12 (92.3%)
Intracranial hypertension	—	6 (28.5%)	—	—	1 (12.5%)	5 (23.8%)
Seizure	—	18 (85.7%)	—	—	7 (87.5%)	5 (23.8%)
Cyst stage and burden						
Vesicular cyst(s)	—	19 (90.4%)	—	—	6 (75.0%)	13 (100%)
Single	—	5 (26.3)	—	—	4 (66.6%)	1 (7.6%)
Multiple	—	14 (73.6%)	—	—	2 (33.3%)	12 (92.3%)
Colloidal/nodular cyst(s)	—	7 (33.3%)	—	—	3 (37.5%)	4 (30.7%)
Single	—	5	—	—	3	2

		(71.5%)			(100.0%)	(50.0%)
Multiple	—	2 (28.5%)	—	—	0	2 (50.0%)
Calcified cyst(s)	—	11 (47.8%)	—	—	3 (37.5%)	8 (38.0%)
Single	—	2 (18.1%)	—	—	1 (33.3%)	1 (12.5%)
Multiple	—	9 (81.8%)	—	—	2 (66.6%)	7 (87.5)
CSF analysis						
Protein, >30 mg/dL	19 (52.7	11 (52.3%)	8 (53.3%)	P=1.0	2 (25.0%)	8 (61.5%)
Cell count, >5 cells/mcL	21 (58.3)	15 (71.4%)	6 (40.0%)	P=.06	2 (25.0%)	11 (84.6%)
Glucose, <50 mg/dL	12 (33.3	10 (47.6%)	2 (13.3.5%)	P=.13	1 (12.5%)	11 (84.6%)

* Control diagnoses are included in the methods section and figure 3

** Includes definitive P-NC by **the previous validated criteria (Carpio et al, 2016)**.

*** Includes participants with definitive EP-NC plus definitive P-NC/EP-NC by **the previous validated criteria (Carpio et al, 2016)**. Of these participants, 9 (69%) had subarachnoid cyst(s), and 7 (54%) had intraventricular cyst(s).

Highlights

- Autoantibodies in the CSF from NC patients were detected by quantitative immunoblot
- Autoantibodies are present in the CSF from cases with extraparenchymal NC
- Autoantibody and secreted glycoprotein HP10 levels were strikingly correlated
- Nine corresponding autoantigens were provisionally identified by mass spectroscopy
- Autoantibodies are highly reduced in the CSF from cases with parenchymal NC

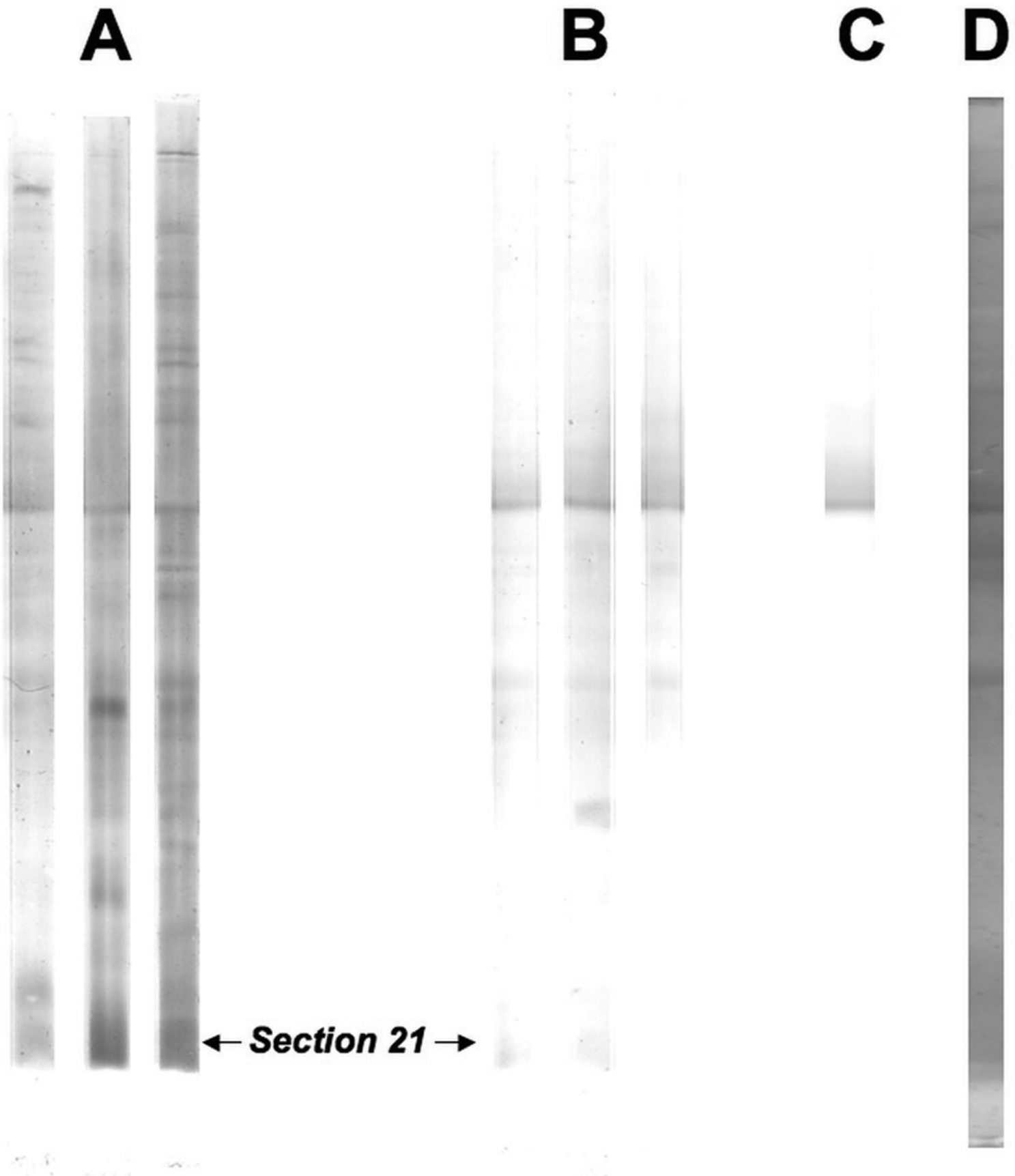

Figure 1

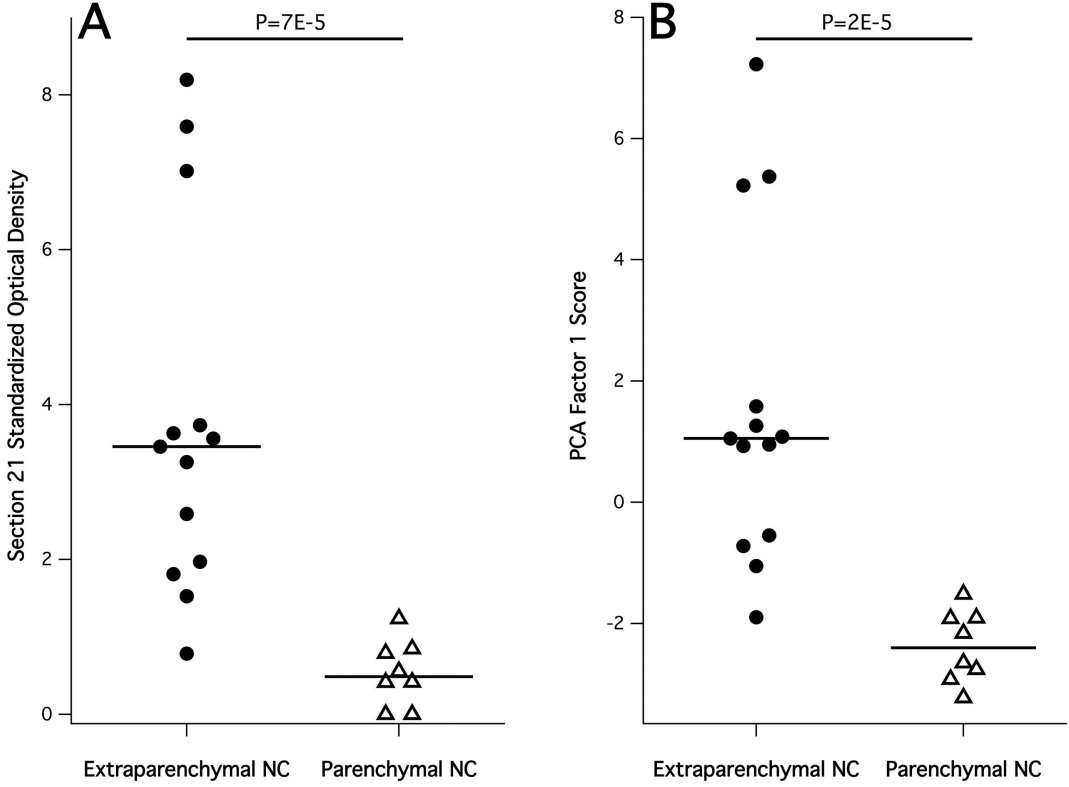
A
P=7E-5
Section 21 Standardized Optical Density
8
6
4
2
0
Extraparenchymal NC
Parenchymal NC
B
P=2E-5
PCA Factor 1 Score
8
6
4
2
0
-2
Extraparenchymal NC
Parenchymal NC

Figure 2

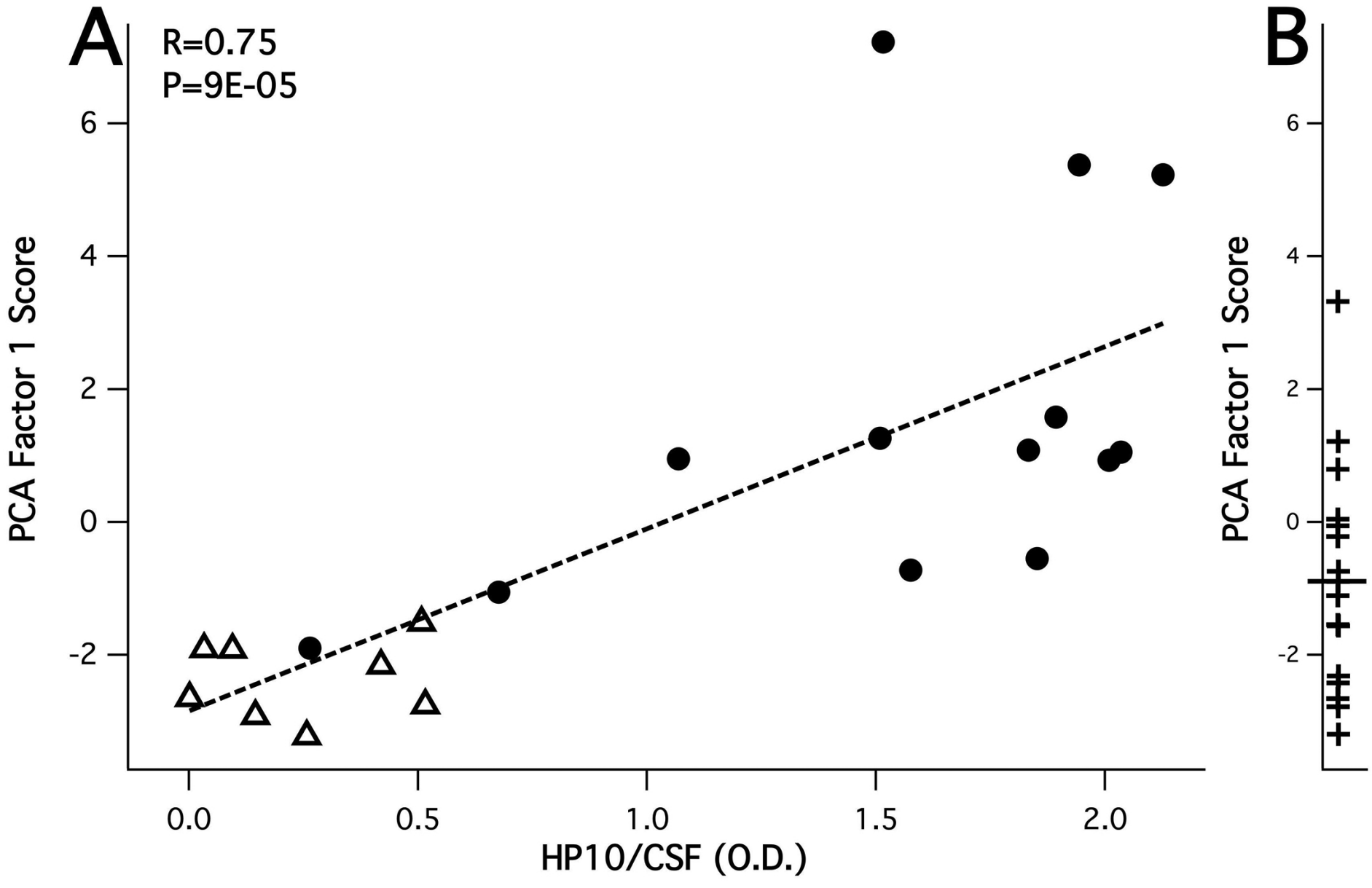
A
R=0.75
P=9E-05
PCA Factor 1 Score
6
4
2
0
-2
0.0
0.5
1.0
1.5
2.0
HP10/CSF (O.D.)
B
PCA Factor 1 Score
6
4
2
0
-2

Figure 3

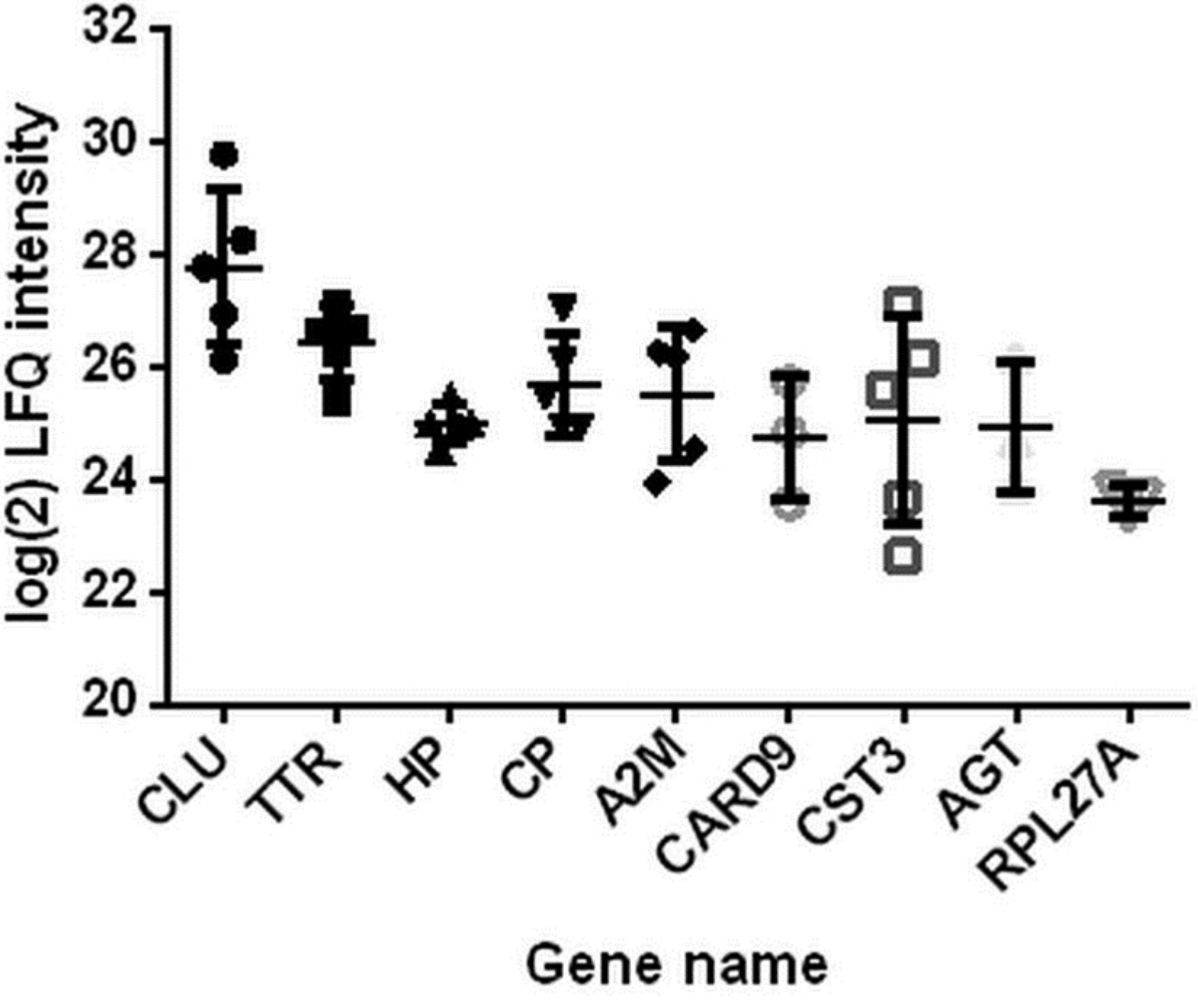
log(2) LFQ intensity
32
30
28
26
24
22
20
CLU
TTR
HP
CP
A2M
CARD9
CST3
AGT
RPL27A
Gene name

Figure 4