Received: 21 May 2021 | Revised: 15 November 2021 | Accepted: 16 November 2021

DOI: 10.1111/epi.17139

Epilepsia®

RESEARCH ARTICLE

Increased expression of complement components in tuberous sclerosis complex and focal cortical dysplasia type 2B brain lesions

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Funding information
V.-E.G. was funded by a grant of the Austrian Epilepsy Society dedicated to M.F. and by the German Tuberous Sclerosis Association (T.S., V.-E.G.). The research leading to these results has also received funding from the European Union's Seventh Framework Program (FP7/2007-2013) under grant agreement 602391 (EPISTOP; E.A.,

Abstract

Objective: Increasing evidence supports the contribution of inflammatory mechanisms to the neurological manifestations of epileptogenic developmental pathologies linked to mammalian target of rapamycin (mTOR) pathway dysregulation (mTORopathies), such as tuberous sclerosis complex (TSC) and focal cortical dysplasia (FCD). In this study, we aimed to investigate the expression pattern and cellular distribution of the complement factors C1q and C3 in resected cortical tissue of clinically well-characterized patients with TSC and FCD2B.

Methods: We applied immunohistochemistry in TSC ($n = 29$) and FCD2B ($n = 32$) samples and compared them to autopsy and biopsy controls ($n = 27$). Furthermore, protein expression was observed via Western blot, and for descriptive colocalization studies immunofluorescence double labeling was performed.

Victoria-Elisabeth Gruber and Mark J. Luinenburg contributed equally.

M.F., A.M., T.S.); the Dutch Epilepsy Foundation (project number 2020-02; A.M., M.J.L., J.v.S.); the ZonMw Translational Research Program (95105004); and the European Union's Horizon 2020 WIDESPREAD-05-2020–Twinning, EpiEpiNet (grant agreement 952455; E.A.).

Results: Protein expression for C3 was significantly upregulated in TSC and FCD2B white and gray matter lesions compared to controls. Staining of the synaptic vesicle protein synaptophysin showed a remarkable increase in the white matter of both TSC and FCD2B. Furthermore, confocal imaging revealed colocalization of complement factors with astroglial, microglial, neuronal, and abnormal cells in various patterns.

Significance: Our results demonstrate that the prominent activation of the complement pathway represents a common pathological hallmark of TSC and FCD2B, suggesting that complement overactivation may play a role in these mTORopathies.

KEYWORDS

complement, cortical development, epilepsy, focal cortical dysplasia, inflammation, tuberous sclerosis complex

1 | INTRODUCTION

Tuberous sclerosis complex (TSC) is a rare autosomal dominant multisystem disease caused by mutations in either hamartin (TSC1) or tuberin (TSC2), key regulators of the mammalian target of rapamycin (mTOR) complex 1.[1] Mutations in these two genes are responsible for the pathological constitutive activation of the mTOR pathway, leading to the development of benign tumors in several organ systems, including subependymal giant cell astrocytoma, cortical tubers, and neuronal migration lines in the brain.[2] A pathology that is both genetically and morphologically similar is focal cortical dysplasia type 2B (FCD2B), where somatic and germline mutations in genes of the mTOR pathway have been reported.[3] Recently, these malformations have been combined under the term "mTORopathies."[4] The most prevalent neurologic symptom in mTORopathy patients is medically intractable epilepsy.[3,4]

Key players of the immune system, such as the classical complement system, have become increasingly associated with epilepsy.[5] Although complement factors are synthesized mainly by hepatocytes in the liver, it was shown that cells of the central nervous system such as glial cells and neurons can also locally produce complement components.[6] There is evidence of complement activation in several neurodegenerative diseases, such as amyotrophic lateral sclerosis and Parkinson disease.[7,8] Interestingly, the classical complement system was discovered to be relevant for neurodevelopment, specifically the modification of synaptic connectivity in physiological and pathological conditions. In the developing visual cortex, excessive synapses are eliminated to establish mature patterns of neuronal connections by C1q-C3 signaling via microglial phagocytosis, whereas during postnatal development, an influence on maturation of cortical and hippocampal neurons was observed.[9,10] Although critical for development, an accumulation of the classical complement factors may induce pathologic synapse loss by recruitment of microglia to phagocytose synapses in diseases such as Alzheimer disease, schizophrenia, and multiple sclerosis.[11–13] This suggests that phagocytosis may be responsible for the observed loss of synaptodendritic structures in epilepsy.[14,15] In terms of human and experimental epilepsy, aberrant complement activation and mRNA levels of complement molecules were reported.[5,16]

Key Points

- We were able to demonstrate a prominent activation of the classical complement in FCD2B and TSC cohorts
- Close proximity of complement factors with cellular elements could indicate a pathological mechanism behind the observed loss of synapses in FCD2B as well as TSC lesions

Increased protein levels of classical complement factors were also observed in brain tissue from FCD patients.[17,18] Induction of the complement system has also been implicated in the pathology of TSC.[19] Recent research has demonstrated that mRNAs encoding components of the complement system are significantly elevated in TSC brain tissue.[20] Interestingly, activated microglia were shown to promote induction of neurotoxic reactive A1 astrocytes through secretion of interleukin (IL)-1α, tumor necrosis factor (TNF), and the complement factor C1q. A1 reactive astrocytes (C3+) fail to promote neuronal survival, outgrowth, and synaptogenesis and may therefore help to drive death of neurons and oligodendrocytes.[21] Consequently, the deregulation of the classical complement system as well

as activation of A1 astrocytes may represent a link between epilepsy and the displayed cognitive impairment observed in these patients.

To identify a potential role for the involvement of the classical complement in cognitive function, the objectives of this study were to (1) determine the protein levels of C1q along with the cleavage products of C3 by immunohistochemistry (IHC) and Western blotting as a measure of the classical pathway activation in resected lesional brain tissue from patients with FCD2B and TSC, (2) determine A1 (C3+) astrocyte activation in our patient cohort, and (3) observe possible opsonization of synapses resulting in unwanted synaptic loss.

2 | MATERIALS AND METHODS

2.1 | Samples

Brain samples were derived from all pediatric patients (age range = 0–18 years) with malformations of cortical development and pharmacoresistant epilepsy who underwent presurgical evaluation and surgery at their respective medical centers. Corresponding formalin-fixed and paraffin-embedded (FFPE) resections containing white matter (WM) and gray matter (GM) were included. In our study, we compared TSC ($n = 29$) and FCD2B ($n = 32$) cases to relatively normal brain tissue resected from age- and localization-matched autopsy ($n = 19$) and biopsy tissue ($n = 8$). We considered tissue to be "control tissue" if histology showed no tumor cell infiltration and patients did not suffer from any history of seizures or other neurological disease. Following these criteria, we used normal-appearing tissue of the following diagnoses: neglectable reactive changes such as mild astroglioses, inflammation or necrosis, and tumor tissue (glioma, glioblastoma, oligoastrocytoma, and oligodendroglioma). All applied biopsy tissue was derived from temporal ($n = 5$), frontal ($n = 2$), or occipital lobe ($n = 1$).

We included 24 TSC and eight autopsy cases from the Neurobiobank AMC Amsterdam. Five TSC, 32 FCD2B, eight biopsy, and 11 autopsy cases from the Neurobiobank of the Division of Neuropathology and Neurochemistry, Department of Neurology of the Medical University of Vienna were used. The Amsterdam Biobank cohort contained FFPE resected brain material from six TSC patients from Erlangen (Department of Neuropathology, University Hospital Erlangen, Erlangen, Germany), one TSC patient from Lisbon (Department of Neurology, Santa Maria Hospital, Lisbon, Portugal), and 16 TSC patients from Utrecht (Department of Pathology, University Medical Center Utrecht, Utrecht, the Netherlands).

All procedures received prior approval by the local ethics committee of the contributing medical centers and were in accordance with the guidelines for good laboratory practice of the European Commission.

2.2 | Immunohistochemistry

Postsurgically, the paraffin sections were routinely processed for histopathology. The following antibodies and dilutions were applied on 3-μm tissue sections mounted on negatively charged glass slides: C1q (Dako, A0136, 1:200), C3c (Dako, A0062, 1:100), C3d (Dako, A0063, 1:400), synaptophysin (Syn; Dako, DAK-SYNAP, 1:500), SMI31 (Covance, SMI31R, 1:25 000), SMI32 (Sternberger, 1:1000), vimentin (Dako, V9, 1:50), and Map2 (Millipore, AP20, 1:8000). Tissue was pretreated with either proteinase K (C1q, C3c, C3d) or pH6 (Syn, Map2, SMI32, SMI31). As a detection system, the EnVision FLEX+ kit (Dako) was utilized and diaminobenzidine as chromogen, executed either with an autostainer (Dako) or via cover plates (Thermo Fisher Scientific glass cover plates). The paraffin sections were counterstained with hematoxylin.

2.3 | Region of interest-based approach for slide analysis

Quantification of the stained paraffin sections was performed applying a recently published protocol by Scholl et al.[22]

In brief, the slides were scanned at 40× magnification using NDPview software (Hamamatsu, NanoZoomer) in Vienna and Olympus dotSlide system (v2.5; Olympus) in Amsterdam. Scans were exported as .TIFF files and transferred to ImageJ (v64, open source). Subsequently, WM and GM regions of interest were selected based on myelin (Nissl–Luxol fast blue), balloon/giant cell (vimentin), and dysmorphic neuron (SMI32, NeuN, Map2) staining. An optimal threshold was chosen for every antibody to distinguish between positive staining and background. The following thresholds were selected: C1q (Vienna cohort: WM, 0–125; GM, 0–100; Amsterdam cohort: WM, 0–170; GM, 0–150), C3c (Vienna cohort: WM, 0–115; GM, 0–140; Amsterdam cohort: WM, 0–150; GM, 0–170), C3d (Vienna and Amsterdam cohorts: WM, 0–160; GM, 0–160), Syn (Vienna cohort: WM, 0–130; GM, 0–100; Amsterdam cohort: WM, 0–200; GM, 0–210), and SMI31 (Vienna cohort: WM, 0–210; Amsterdam cohort: WM, 0–210).

2.4 | Western blotting

Western blotting was performed to determine the complement factor levels between epilepsy patient groups and controls. It

TABLE 1 Patient data overview

Type	*n*	Sex (F:M)	Age (±SD)	Frequency (days/weeks/months)	Autism (yes/no)	Mutations
Autopsy & biopsy	27	16:11	15 (11)	—	—	—
TSC	29	12:16[a]	12 (10)	15/4/—[a]	8/8[a]	9 *TSC1*/13 *TSC2*
FCD2B	32	10:22	8 (10)	17/7/2[a]	3/3[a]	1 *NPRL3*

Note: n refers to the number of cases included. Unit of age is mean years. Seizure frequency is split into daily, weekly, and monthly. Autism is split into yes or no.

Abbreviations: F, female; FCD2B, focal cortical dysplasia type 2B; M, male; *NPRL3*, nitrogen permease regulator-like 3; TSC, tuberous sclerosis complex; *TSC1/2*, tuberous sclerosis complex 1/2 (hamartin/tuberin).

[a] Indicates missing data.

could only be performed when frozen material was available. Lesional samples from 13 frozen tissues—from four FCD2B patients (5.7 ± 5.9 years), five TSC patients (12.2 ± 5.8 years), and four autopsy controls (11.3 ± 5.6 years)—were analyzed. The tissue was lysed using RIPA lysis buffer (Upstate, 20–188) supplemented with phosphatase 2,3 (Sigma-Aldrich, P5726, P0044) and protease inhibitor cocktail (Sigma-Aldrich, P8340). The total protein content was determined via bicinchoninic acid assay (Thermo Fisher Scientific) in triplicate. Equal amounts of total protein (15 μg) were dissolved in sample buffer (Thermo Fisher Scientific, 4 × LDS Sample Buffer) containing 100 mmol·L^{-1} dithiothreitol and subsequently boiled at 70°C for 10 min. Proteins were separated on gels (NuPAGE 12%, Bis-Tris Mini Protein Gel) and run for 80 min at 100 V. Tank blotting was applied using polyvinylidene difluoride membranes at 100 V for 60 min. After blocking the nonspecific binding sites with 5% bovine serum albumin (BSA) for 1 h, the membranes were incubated with the primary antibodies (GAPDH, 1:10 000, Sigma-Aldrich, A3854; C1q, 1:3000, Dako, A0136; C3d, 1:4000, Dako, A0063) overnight at 4°C. After washing steps with 1 × Tris-buffered saline with Tween, secondary antibodies (P044701 and P044801, Dako) were utilized for 1 h. Enhanced chemiluminescence (ECL) solution (Thermo Fisher Scientific, Pierce ECL Western Blotting Substrate) was used for development of membranes. The signal was captured with an ImageQuant LAS 4000 imager (GE Healthcare), and bands were quantified using ImageJ software (v64, open source).

2.5 | Immunofluorescence staining and imaging

Paraffin-imbedded sections were deparaffinated in xylene and 96% ethanol. Antigen retrieval was performed by boiling the slides in citrate buffer (10 mmol·L^{-1} sodium citrate, pH 6.0) for 10 min in an autoclave, cooling them down, and washing them with phosphate-buffered saline (PBS). Slides were then blocked in 5% BSA, .1% Triton-X100 for 30 min at room temperature (RT). Slides were incubated with a primary antibody mix containing either 1:200 C1q (Dako, A136)–1:2000 glial fibrillary acidic protein (GFAP; Sigma-Aldrich, G-3893), 1:200 C1q (Dako, A136)–1:1000 microtubule-associated protein 2 (Map2; Sigma-Aldrich, M4403), 1:200 C1q (Dako, A136)–1:100 human leukocyte antigen–DR isotype (HLA-DR; Dako, CR3/43), 1:2000 GFAP (Sigma-Aldrich, G-3893)–1:100 C3c (Dako, A062), or 1:2000 GFAP (Sigma-Aldrich, G-3893)–C3d (Dako, A063). All primary incubations were performed for 1 h at RT followed by an overnight incubation at 4°C. Following incubation, the slides were washed three times with washing buffer (PBS, .1% Triton X-100, .5% BSA) for 10 min each. Secondary antibodies conjugated with Alexa Fluor 488 (Invitrogen, A-11011) or 568 (Invitrogen, A-10037) were incubated at 2 μg/ml for 2 h in the dark. Finally, slides were washed three times with washing buffer and three times with PBS, and mounted with Vectashield vibrance with 4,6-diamidino-2-phenylindole (Vector Laboratories, H-1800). Slides were imaged with a SP8 confocal microscope (Leica) with equal settings for each double-stained pair.

2.6 | Statistical analysis

Statistical analysis was performed utilizing SPSS (IBM, v26.0). Graphical data visualization was accomplished in GraphPad Prism (v7). Statistical analysis was performed using nonparametric independent Mann–Whitney *U* test to analyze differences between groups. In general, for the statistical analyses, a confidence interval of 95% was applied . Spearman rho was performed for correlations.

3 | RESULTS

3.1 | Clinical data

In total, we included tissue from 88 patients: 29 cortical tubers from patients with TSC, 32 lesional resections from

FCD2B patients, and autopsy/biopsy tissue of 27 patients (Table 1). Mutations were present in 22 TSC patients (nine *TSC1* and 13 *TSC2*) and in some of the FCD2B patient cohort (one *NPRL3*; Table 1). Autistic behavior was seen in 27.5% of TSC and 9.3% of FCD2B patients.

3.2 | Enhanced classical pathway activation in FCD2B and TSC patient samples

We used immunoblotting to measure the protein levels of C1q and C3 proteins in FCD2B ($n = 4$) and TSC ($n = 5$) patient lesional tissue and compared it to age-matched autopsy tissue ($n = 4$). We found that the protein levels of C1q and C3d shown at the predicted molecular weight of 235 kDa and 125 kDa, respectively, were increased in patients. In parallel, we observed that the protein level of C3c (~45 kDa, alpha chain fragment) was significantly higher in FCD2B and TSC ($p \leq .05$) patient material (Figure 1).

To identify where the products of the classical pathway activation are deposited in the cortical brain tissue of our patient cohort, we performed immunohistochemical staining for C1q and the end products of the C3 activation pathway, C3d and C3c. All positive cells were counted with the automated region of interest (ROI)-based approach and converted to cells/mm^2. Our measurements revealed a significant expression of C3c in the WM of FCD2B ($p \leq .001$) and TSC ($p \leq .001$) samples compared to control (Figure 2). Significantly increased levels of C3d were further demonstrated in lesional WM of FCD2B ($p \leq .000$) and TSC ($p \leq .001$) patients (Figure 2). Interestingly, we found significantly higher amount of C3d+ staining in the lesional GM of FCD2B ($p \leq .000$) and TSC ($p \leq .000$) patients (Figure 3). In general, an overall trend toward enhanced classical complement system was observed in lesional GM of patients (Figure 3).

To visualize possible interaction between complement and the various cells present in the neurological environment, we performed double staining of C1q and C3 with neuronal (Map2), astrocytic (GFAP), and activated microglial (HLA-DR) markers (see Figure 4, Figure S1). These stainings revealed that the microglial marker overlaps with C1q, whereas astrocytes tend to express more C3c and C3d. Moreover, Map2 seems to localize with C1q, showing close proximity at dendrites (Figure 4, Panels 1, 11, 16). Interestingly, we also observed evenly distributed complement factors with astrocytes (Figure 4, Panels 2, 3, 13, 19) and cell membrane foci on the activated microglia (Figure 4, Panels 5, 15, 20). To observe a possible correlation between classical complement factors and abnormal cells, we performed double stainings with a marker for balloon/giant cells (vimentin) and dysmorphic neurons (SMI32) (Figure S2). We found a colocalization of C3 proteins with balloon/giant cells and dysmorphic neurons. In contrast, C1q seems to be expressed in close proximity to abnormal cells of TSC and FCD2B patients (Figure S2). This suggests that there are multiple dimensions regarding complement function within the lesion, most likely orchestrated by cross-signaling between cell types.

In summary, increased levels of C1q and C3 proteins were found in cortical tissues of FCD2B and TSC patients and are expressed across glial cells and abnormal cells in

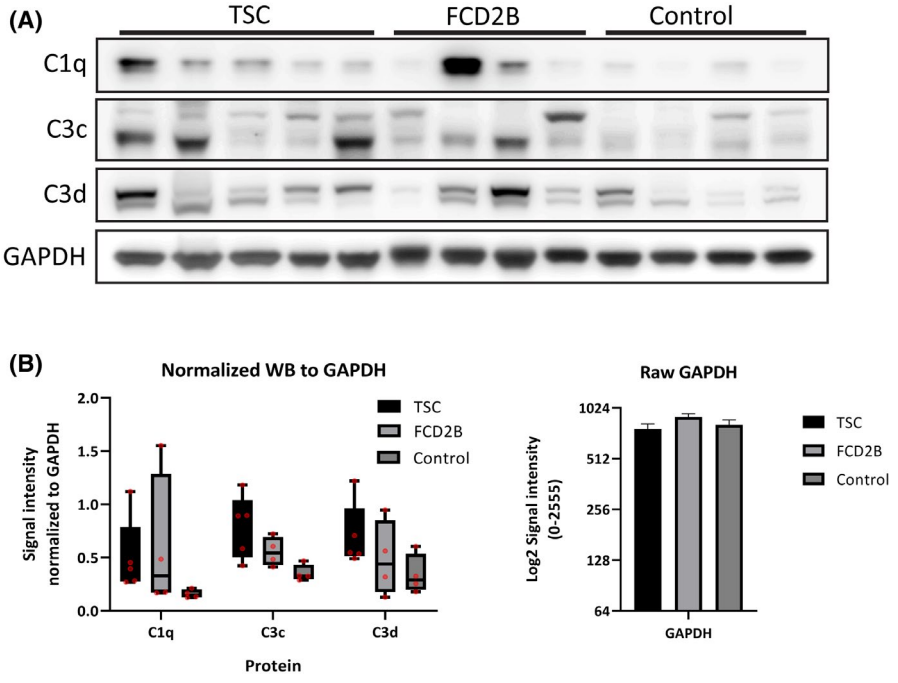

FIGURE 1 Upregulation of classical complement system. (A) Western blot (WB) of C1q, C3c, and C3d in representative control ($n = 4$), tuberous sclerosis complex (TSC; $n = 5$), and focal cortical dysplasia type 2B (FCD2B; $n = 4$) patient samples. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. (B) Quantification of WB by normalized signal intensity. Unpaired *t*-test was used as the statistical test; $^*p < .05$

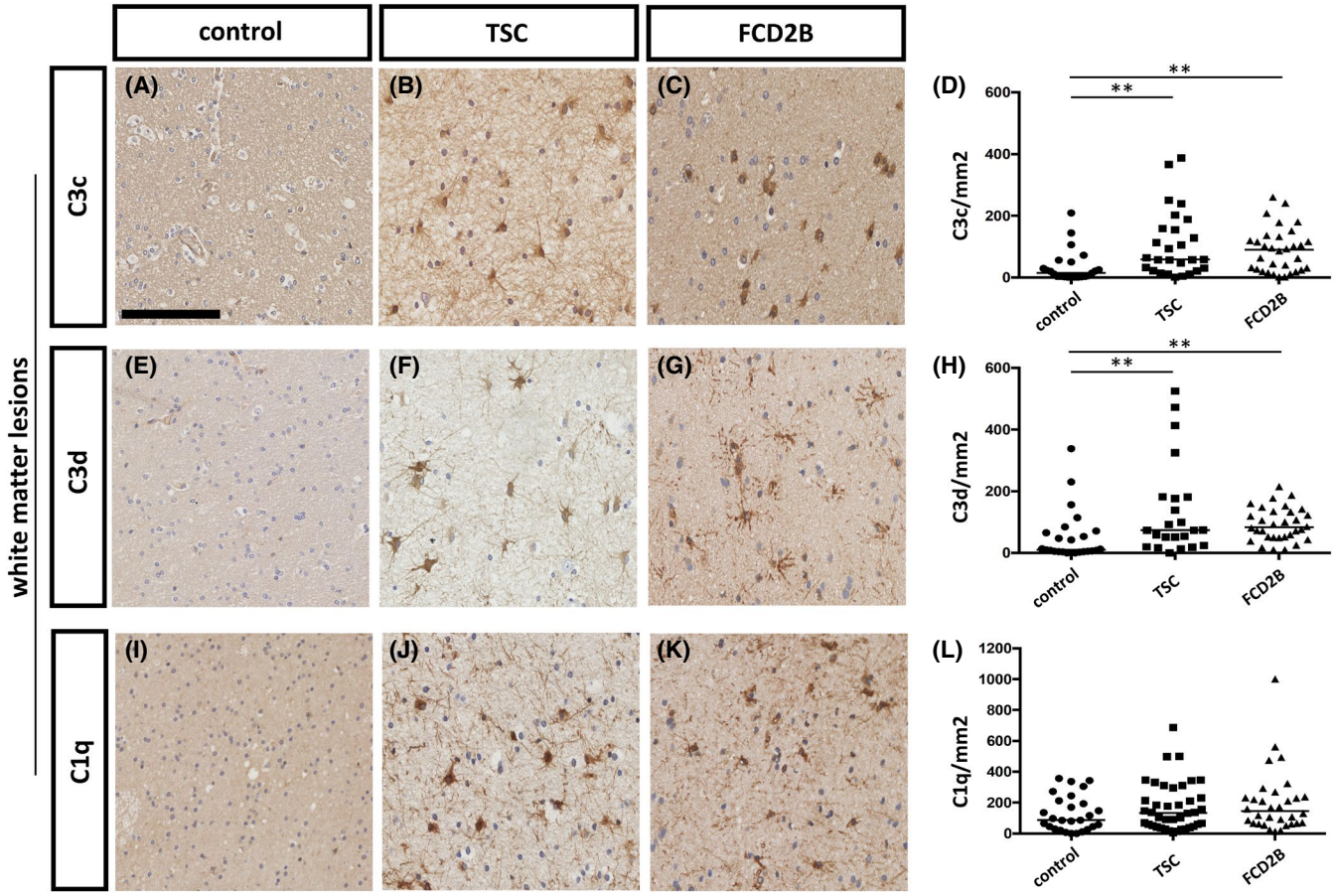

FIGURE 2 Enhanced expression of classical complement factors C3c and C3d in lesional white matter of tuberous sclerosis complex (TSC) and focal cortical dysplasia type 2B (FCD2B) patients. (A–C) White matter C3c staining. (D) Cell number from region of interest (ROI)-based quantification of C3c (controls, $n = 23$; TSC, $n = 27$; FCD2B, $n = 32$). (E–G) White matter C3d staining. (H) Cell number from ROI-based quantification of C3d (controls, $n = 25$; TSC, $n = 24$; FCD2B, $n = 32$). (I–K) White matter C1q staining. (L) Cell number from ROI-based quantification of C1q (controls, $n = 27$; TSC, $n = 27$; FCD2B, $n = 32$). Mann–Whitney *U*-test was applied for statistical analysis; $^{**}p < .001$. Scale bar = 100 μm for all immunohistochemical images

different patterns. Furthermore, C1q colocalizes on neuronal dendrites and around neuronal bodies.

3.3 | Altered Syn content in patients with TSC or FCD2B

We applied immunohistochemistry and quantified the density of Syn+ presynaptic terminals. Lesional Syn quantification of the ROI was digitally assessed and compared to autopsy and biopsy WM and GM. The results in Figure 5 show that Syn staining in control brain WM did not yield a signal, in contrast with that obtained from TSC and FCD2B patients. Our analysis of the intensity of the staining revealed a level of .04% in the control group (Figure 5D). In comparison, the intensity for TSC and FCD2B reached a median level of 16% and 21% (Figure 5D; $p \leq .000$ and $p \leq .000$ vs. control, respectively). Furthermore, in lesional GM of FCD2B patients, less Syn staining intensity (78%) was observed compared to controls (89%, $p \leq .001$; Figure S3).

3.4 | Correlations

Further analysis of the observed enhanced classical complement activation as well as Syn pathology and clinical data (Table 1) was performed. A statistical correlation between the incidence of autism and increased Syn expression in the lesional WM of TSC and FCD2B patients was observed (Spearman rho, $p = .4$). Interestingly, a higher seizure frequency correlated with increased lesional WM C3d ($p = .4$), C3c ($p = .4$), and Syn ($p = .7$). Also, increased C3d staining in the lesional GM correlated with higher seizure frequency ($p = .5$). In the lesional GM, reduced Syn staining correlated with increased seizure frequency ($p = .3$).

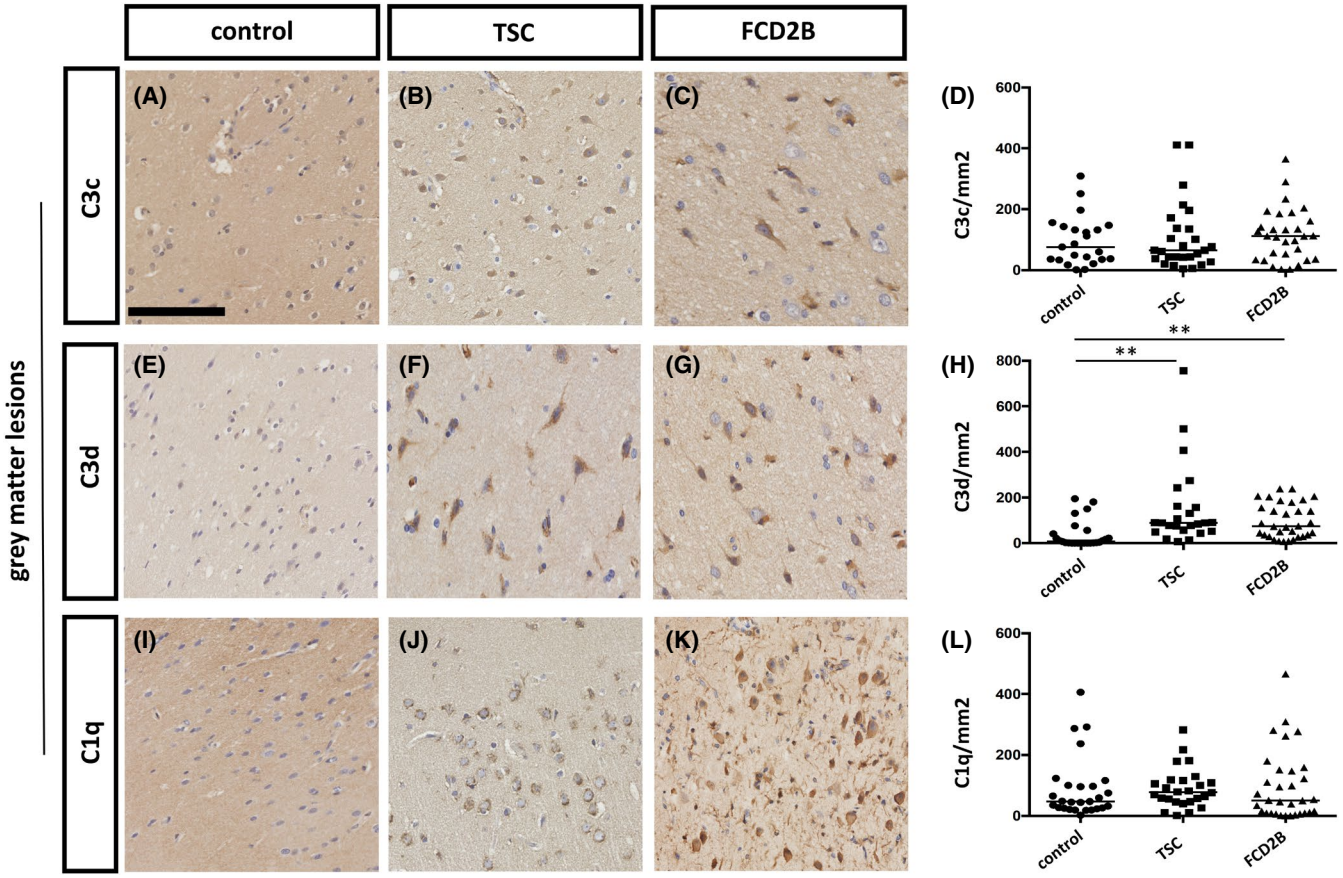

FIGURE 3 Upregulation of C3d staining in lesional gray matter of tuberous sclerosis complex (TSC) and focal cortical dysplasia type 2B (FCD2B) patients. (A–C) Gray matter C3c staining. (D) Cell number from region of interest (ROI)-based quantification of C3c (controls, $n = 23$; TSC, $n = 27$; FCD2B, $n = 30$). (E–G) Gray matter C3d staining. (H) Cell number from ROI-based quantification C3d staining (controls, $n = 25$; TSC, $n = 24$; FCD2B, $n = 32$). (I–K) Gray matter C1q staining. (L) Cell number from ROI-based quantification of C1q (controls, $n = 27$; TSC, $n = 26$; FCD2B, $n = 32$). Mann–Whitney *U*-test was applied for statistical analysis; $^{**}p < .001$. Scale bar = 100 μm for all immunohistochemical images

4 | DISCUSSION

Neuroinflammation has been implicated in many neurological conditions, such as temporal lobe epilepsy (TLE),[5,23] traumatic brain injury,[5,23] and status epilepticus.[24] In the latter, it also correlates with the frequency of seizures. In our study, we quantified the amount of C1q and C3 in GM and WM lesions of TSC and FCD2B patients, where we found an upregulation of classical complement factors, especially of the protein C3d. This is supported by the previously established observations that complement and epilepsy are intertwined. Moreover, our results suggest that the connection also extends to the mTORopathies, as suggested in a previous study of TLE in FCD2B.[17] This, however, was never quantified in a large cohort. Thus, our data extend the hypothesis that complement activation is a result of epileptogenic activity seen in a wide variety of pathologies, of both environmental and genetic origin. Additionally, we introduce a double-stain fluorescent confocal microscopy to expand these observations in relation to cellular identity and complement localization.

We observed that, although a trend was seen in the upregulation of C1q, the downstream cascade elements C3c and C3d were significantly upregulated in comparison to control tissue. C1q is known to be expressed primarily by microglia,[25] whereas C3 seems to be more dominant in the astrocytes[25] and oligodendroglia.[26] The receptor of C3a expressed by microglia is activated by secreted C3.[27] In comparison, an in vivo study showed that a mix of TNFα, IL-1β, and C1q was sufficient to produce C3+ reactive astrocytes.[21] This indicates a complicated crosstalk between microglia and astrocytes to facilitate the inflammatory state. In addition to cellular expression, C1q and C3 have also been shown to colocalize with immature neuronal dendrites, and C1q itself has been reported to underlie synaptic pruning.[28] Some evidence has been collected that supports the notion of excessive synaptic pruning in epilepsy, as well as inflammation-related diseases such as multiple sclerosis

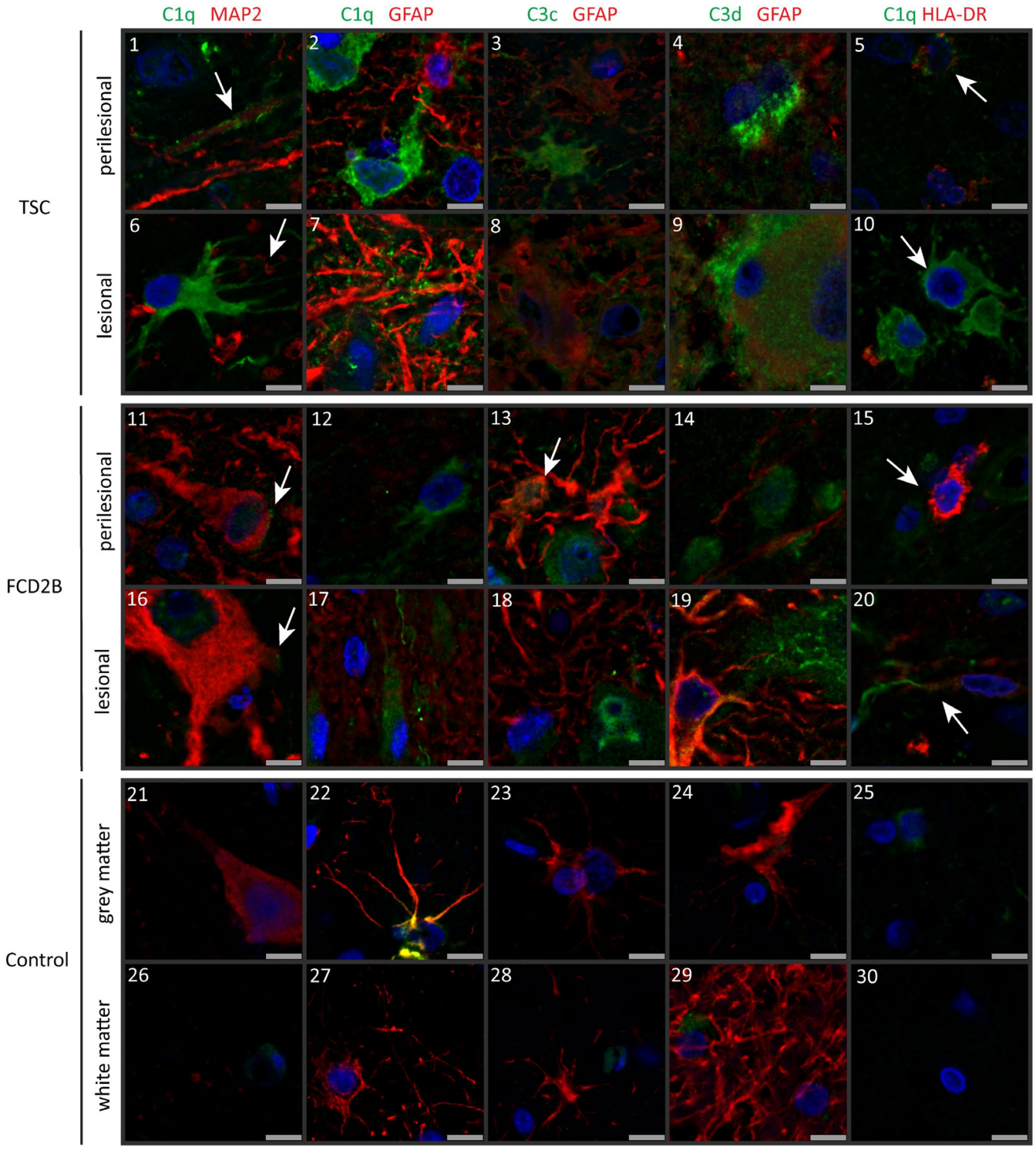

FIGURE 4 Descriptive complement double staining with various cell markers at ×63. Areas per double staining were divided between pathologies (tuberous sclerosis complex [TSC] and focal cortical dysplasia type 2B [FCD2B]) and between lesional and perilesional. For control tissue, the division was made between white and gray matter. Map2 (microtubule-associated protein 2) indicates neuronal cells, GFAP (glial fibrillary acidic protein) represents (activated) astrocytes, and HLA-DR (human leukocyte antigen–DR isotype) shows activated microglia. Highlighted elements (white arrows): (1) Map2+ dendrites of neurons are coated by C1q; (5) C1q colocalizes with activated microglia; (6) C1q+ cell interacting with Map2+ debris; (10) C1q+ cells can be independent of microglial markers; (11) perilesional neuron in close proximity with C1q; (13) C3+ astrocytes in close proximity with C3+ cells; (15) microglia in close proximity to C3+ cells; (16) dysmorphic neuron with dendrite tagged by C1q; (20) activated microglia interacting with C1q+ fibers. Scale bars = 10 μm

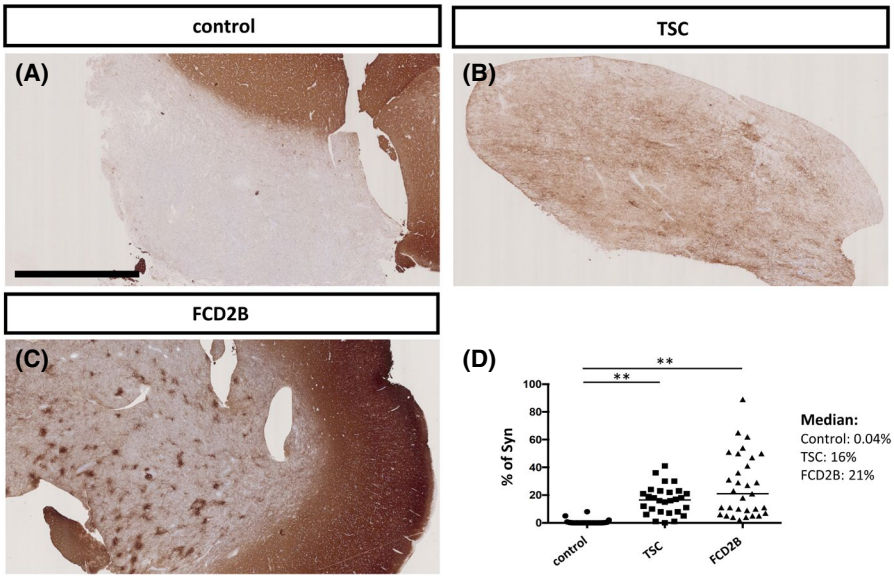

FIGURE 5 Expression of synaptophysin (Syn) in lesional white matter of tuberous sclerosis complex (TSC) and focal cortical dysplasia type 2B (FCD2B) patients. (A–C) White matter Syn staining. (D) Region of interest quantification of Syn staining as a percentage (controls, $n = 26$; TSC, $n = 28$; FCD2B, $n = 31$). Mann–Whitney *U*-test was applied for statistical analysis; $^{**}p < .001$. Scale bar = 2.5 mm for all immunohistochemical images

and Alzheimer disease.[11,13] Psychiatric diseases are also known to result in deregulated complement activation, especially schizophrenia.[29] Pruning C1q has been known to induce apoptotic processes,[30] whereas inhibition of C1q preserves synaptic integrity in a glaucoma mouse model.[31]

Excessive pruning through the complement system could explain the loss of neurons often seen in FCD and TSC.[32] We showed a significantly lower staining of Syn in the lesional GM of the patient cohort, and C1q IHC staining indicated neuronal localization of C1q. In contrast, IHC staining of C1q and C3 in the lesional WM of patients pointed to astrocytes as a main source of staining signal. This shows a difference between the expression patter of complement factors and the loss of Syn in lesional GM and WM.

Moreover, our double labeling supports this hypothesis due to the colocalization of C1q with Map2. Interestingly, C1q+ cells could be seen independently of HLA-DR staining. We also encountered localization of C3c and C3d on dysmorphic cells in focal patterns, often in close proximity of C3+ reactive astrocytes. This focal pattern was also observed in a previous study on multiple sclerosis,[13] and reactive astrocyte function has been related to elimination of cortical neurons and mature oligodendrocytes in in vivo studies by Liddelow et al,[21] two cell types that are diminished in mTORopathies.[32,33] However, one limitation of this study is that the colocalization study was of descriptive nature; hence, no strong conclusions can be formed on their results. Furthermore, complement expression across the various types of neural cells and their exact biological function is still under investigation.

FCD and TSC pathologies are hallmarked by their mTOR hyperactivation, and its resulting neuroinflammation is implicated as one of the major driving forces in the associated epileptogenesis.[34,35] Controversially, cytokine production is generally decreased upon mTOR hyperactivation in a host of immune cells,[17,36,37] in contrast with the increase of inflammatory cytokines seen in mTORopathies.[17,18,20,37] Interestingly, Zhao et al. report that reactivelike microglia promote epileptogenesis independently of the inflammatory response in TSC 1 −/− mouse models,[38] which resulted in an increase of microglia infiltration, reduction of neuronal synapses, and proliferation of astrocytes. Overall, this could provide two different paths toward microglia-directed epileptogenesis: inflammation factor dependent, as seen with direct insults, and factor independent, as a result of genetic aberrations such as TSC and FCD.

To conclude, we present an upregulation of classical complement factors in mTORopathies, presumably due to their inherent pathological properties, such as cortical malformation and mTOR dysregulation. An increase in classical complement factors correlates with seizure frequency, hence indicating a worsening of the pathology due to epilepsy.

Our descriptive colocalization shows close proximity of C1q with dendrites, which could indicate a mechanism leading to reduction of synapses in GM lesions. Furthermore, we observed a wide variety of staining patterns that could indicate intrinsic biological regulation and possible synaptic degradation through reactive astrocytes and complement-mediated pruning.

ACKNOWLEDGMENTS

The authors thank the team at the Epilepsy Monitoring Unit at the Department of Pediatrics at the Medical University of Vienna for their support in preparing clinical data and the neuropathology team at the Division of Neuropathology and Neurochemistry, Department of

Neurology at the Medical University of Vienna for adequate processing and storage of tissue. The authors would also like to thank all members of the TSC brain bank for their cooperation (Service d' Anatomie Pathologique, CHI de Creteil and Inserm U676, Hospital Robert Debre, Paris, France: H. Adle-Biassette; Department of Pediatrics/Institute of Neurology/Department of Neurosurgery, Medical University of Vienna, Vienna, Austria: T. Czech; Department of Neuropathology, John Radcliffe Hospital, Oxford, UK: C. Kennard; Department of Anatomic Pathology Sciences, Università Sapienza, Rome, Italy: M. M. Antonelli, F. Giangaspero; Institute of Neuropathology, Westfälische Wilhelms-Universität Münster, Münster, Germany: W. Paulus; Department of Neuropathology, Centro Hospitalar Lisboa Norte, EPE, Lisbon, Portugal: J. Pimentel; Department of Human Pathology and Oncology, University of Florence and Division of Neurosurgery, Anna Meyer Pediatric Hospital, Florence, Italy: A. M. Buccoliero, F. Giordano).

CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

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How to cite this article: Gruber V-E, Luinenburg MJ, Colleselli K, Endmayr V, Anink JJ, Zimmer TS, et al. Increased expression of complement components in tuberous sclerosis complex and focal cortical dysplasia type 2B brain lesions. Epilepsia. 2022;63:364–374. https://doi.org/10.1111/epi.17139