Plasma biomarker profile and clinical correlations in Tuberous Sclerosis Complex: findings from an adult cohort

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Abstract

Background and objectives: Tuberous Sclerosis Complex (TSC) is a neurocutaneous syndrome and a model of mTORpathy. Its neurological manifestations include epilepsy and TAND (*TSC-associated neuropsychiatric disorders*), whose pathophysiological mechanisms and long-term prognosis are yet partly unknown. Based on clinical/paraclinical similarities with fronto-temporal dementia, TSC has been proposed as an 'infantile tauopathy'. However, other mechanisms involving both neurons and glia could contribute. We assessed a wide set of biomarkers of neurodegeneration and astrocytopathy in the plasma of adult TSC patients to define the biomarker profile of TSC and investigate the potential correlations with the clinical-radiological features.

Methods: Thirty-one patients (20 females; median age 31 years, range 15-66 years) with clinical and/or genetic diagnosis of TSC were consecutively enrolled. By using the ultra-sensitive Single Molecule Assay (Simoa™) technique, the plasma levels of the following biomarkers were compared between the patients and 38 age- and sex-matched healthy controls (HC): total Tau (tTau), phosphorylated Tau181, $Abeta_{40}$, $Abeta_{42}$, Neurofilament Light Chain (NfL), and Glial Fibrillary Acid Protein (GFAP). Demographics, clinical features and radiological findings were collected and analyzed.

Results: GFAP plasma levels were significantly higher in TSC patients than in HC (206.03±233.23 vs 53.08±32.88 pg/mL, $p<0.001$), regardless of their genotype. NfL concentration was increased in the whole study population (TSC: 6.31±5.32 vs HC: 4.04±1.74 pg/mL, $p=0.042$), but when stratifying according to the genotype, the significance was confirmed only in TSC2 ($p=0.039$). Similarly, tTau was increased in the TSC2 group alone ($p=0.043$). Interestingly, GFAP appeared correlated with the disease clinical ($\rho=0.498$, $p=0.005$) and radiological severity ($\rho=0.417$, $p=0.001$). In addition, it was significantly higher in patients with a history of epileptic spasms ($p<0.0001$), moderate-severe intellectual disability ($p=0.040$) and autism spectrum disorder ($p\leq0.001$).

Discussion: Our study documented a significant increase of GFAP plasma concentration in TSC adult patients, apparently correlated with the severity of their neurological phenotype. These findings support the central role of astrocytopathy in the pathophysiology of TSC. Most importantly, our work suggests that plasma GFAP could be a candidate prognostic biomarker in TSC.

Introduction

Tuberous Sclerosis Complex (TSC) is a neurocutaneous syndrome resulting from the loss-of-function mutation of either the TSC1 or TSC2 gene, leading to the dysregulation of the mammalian target of rapamycin (mTOR) pathway, ubiquitously involved in crucial functions such as protein synthesis, cell proliferation and metabolism, cytoskeleton stability and autophagy, which accounts for TSC manifold features[1].

Epilepsy is one of the earliest and most dramatic manifestations of this rare condition, that has become a sort of "model disease" in this context: indeed, the recent evidence that epilepsy onset can be prevented or delayed in TSC patients[2] has possibly brought forward a definite change in the whole paradigm of epilepsy treatment, shifting from anti-seizure to anti-epileptogenic medications. Another core symptom is the so-called TAND (i.e. *TSC-associated neuropsychiatric disorders*)[3], a complex and heterogeneous spectrum of cognitive, behavioral and psychiatric alterations that heavily affects the quality of life of both children and adults living with this disease[4,5].

Despite intense research, the features and pathophysiological mechanisms underlying TSC-related epilepsy and TAND are yet to be fully understood, as well as their long-term prognosis. Interestingly, a continuum between TSC and fronto-temporal dementia (FTD) has been recently hypothesized by Liu and colleagues, who found some similarities - in terms of mood/behavioral alterations, executive dysfunctions and language impairment – between these two conditions, and also documented increased levels of phosphorylated tau (pTau) in the cerebrospinal fluid (CSF) of few TSC subjects[6]. A later work by the same author showed 3R/4R pTau accumulation in the brain tissue samples of 9 TSC patients (though much sparser than in FTD)[7], supporting the hypothesis of TSC as an "infantile tauopathy"[8]. Notwithstanding, neuroinflammatory and neurodegenerative processes other than hyperphosphorylated tau accumulation - and equally involving neurons and glia - are likely to contribute to TSC neurological manifestations. Indeed, following the evidence of severe astrogliosis in the epileptogenic cortical tubers, astrocytic alterations have been suspected to play a crucial role in TSC-related epilepsy[9,10], and pre-clinical studies have suggested that they could participate in TAND genesis as well[11].

Based on these premises, for the first time we assessed a wide set of biomarkers of neurodegeneration and astrocytopathy in the plasma of an adult TSC cohort, to define the biomarker profile of TSC and investigate its correlations with the patients' clinical-radiological features.

Methods

Patients' selection and clinical evaluation

Thirty-one adult patients (≥15 years of age) followed at the Epilepsy outpatient clinic and the Centre for Rare Diseases of Policlinico "Umberto I" – "Sapienza" University of Rome, with a confirmed clinical and/or genetic diagnosis of TSC[12], were consecutively enrolled over a 12-month period. The patients' clinical charts were carefully reviewed to obtain information about their family, medical and neurological history: in particular, epilepsy and seizure type (defined according to the 2017 ILAE classification proposal)[13,14], history of epileptic spasms (ES), seizure frequency, number of previous and concomitant ASMs and non-pharmacological treatments were collected and analysed. The patients underwent a thorough neurological examination, including TAND-checklist, MiniMental State Examination (MMSE) or Montreal Cognitive Assessment (MoCA), according to their intellectual level. The Neurological severity score (NSS) was calculated as follows: 1-3 points for intellectual disability (mild, moderate and severe), 1-2 points for epileptic outcome (seizure-freedom or drug-resistance), 2 points for autism spectrum disorder (ASD), 1 point for other psychiatric disorders[15]. All patients also underwent a video-EEG exam (Micromed System Plus, Treviso, Italy; Xltek® EEG, Oakville, Canada) and a high-field (1.5-3 T) brain MRI scan. A modified Radiological Severity Score (RSS) was assigned to each patient, based on a 3-point ordinal scale ranging from 0 to 4, evaluating the burden of cortico-subcortical tubers (1 point if tubers < 3, 2 if tubers > 3), subependymal nodules (SEN, 1 point) and subependymal giant astrocytoma (SEGA, 1 point)[16]. Finally, the overall severity score was calculated as the sum of NSS and RSS.

Thirty-eight age- and sex-matched volunteers were selected as a control group among hospital/university personnel and their family members. Only subjects with normal neurological examination, MoCA score > 26[17] and no history of neurodegenerative disorders, major head trauma, cerebrovascular events, autoimmune brain disorders or genetic syndromes were enrolled as healthy controls (HC).
The main aim of this study was to compare the plasma levels of the following biomarkers in patients and HC: a) neurodegeneration biomarkers: tTau, pTau181, $Abeta_{40}$, $Abeta_{42}$; b) neuroaxonal injury biomarker: Neurofilament Light Chain (NfL), and c) astrocytopathy biomarker: Glial Fibrillary Acid Protein (GFAP). The secondary objective was to investigate the possible correlations between the biomarkers' levels and the patients' demographic, genetic, clinical and radiological features.

Biomarker assessment
Three blood samples were drawn from all the study participants and collected into two 6-mL EDTA tubes and one EDTA-free glass vial for serum. In all the patients, no seizure had occurred for at least 24 hours before the sample collection. The blood samples were initially processed at the "Fondazione European Brain Research Institute (EBRI) Rita Levi-Montalcini" facility by centrifugation at 1600 g for 15 minutes (plasma samples) or 10 minutes (serum samples). Small volumes of plasma and serum were aliquoted into 1.5-2-ml LoBind BD vials (250 uL per vial) and then stored at -80° within 1 h from the collection. Plasmatic protein assays were performed in duplicate for each analysis by using the Single Molecule Assay (Simoa™) technique, SR-X™ Biomarker Detection System from Quanterix, following the specific protocols provided by the used advantage kits (Neurology2-PlexB, Neurology3-PlexA, p-Tau181V2). All the analyses were performed at the Department of Neurosciences of "Istituto Superiore di Sanità" of Rome. The procedure details are included as supplementary material (Supplementary File).

Statistical analysis
Demographic, clinical and laboratory data were transferred into an anonymized electronic database and processed using the Statistical Package for the Social Sciences (SPSS, IBM software inc., Armonk, New York, US) for Windows software, version 27.0. The Shapiro-Wilk test was performed to check the data distribution, resulting in a general non-normal distribution. Data were therefore presented as median (interquartile range [IQR]). Mann-Whitney U test or Kruskal-Wallis test were used to evaluate differences between two or more groups. Categorical variables were presented as frequency (count) and compared across relevant groups using Pearson chi-square or Fisher exact tests. Statistical correlations between the rankings of two continuous variables were assessed with the non-parametric Spearman's test. All tests were two-sided. A p-value of ≤ 0.05 was considered statistically significant.

Standard Protocol Approvals, Registrations and Patient Consents
The present study was approved by the Ethics Committee of "Sapienza" University – Policlinico "Umberto I" of Rome (protocol number 0989/2021). A written informed consent was obtained from all the study participants or their guardians.

Data availability statement
The raw data supporting the conclusions of this paper are available from the corresponding author upon reasonable request.

Results

Patient demographics and general data
Among 31 patients enrolled in the study (20 females; median age 31 years, range 15-66 years), a TSC2 mutation was found in 19 (61.3%) and a TSC1 mutation in 9 (29.0%). Family history was positive for TSC in 11 cases (35.5%). ES were reported in 13 subjects (41.9%) and appeared associated with TSC2 mutation (p=0.032). Twenty-three out of 31 patients (74.2%) had epilepsy at the time of enrolment, including two individuals with Lennox-Gastaut syndrome, and 13/23 (56.5%) met the criteria of drug-resistance[18] (Supplementary Table 1). TAND was diagnosed in 71.0% (n=22) of the study group. The cognitive assessment revealed mild and moderate-severe ID in 10/31 (32.3%) and 11/31 (35.5%) patients, respectively,

and a strong association was observed between severe ID and TSC2 mutation (p=0.012). Anxiety (14/31, 45.2%), behavioural problems (12/31, 38.7%) and ASD (11/31, 35.5%) were the most common neuropsychiatric disorders, followed by obsessive-compulsive disorder (8/31, 25.8%) and depressive mood (6/31, 19.4%). As to the radiological findings, brain MRI scans documented cortico-subcortical tubers in all patients but two (29/31, 93.5%), SENs in 19 (61.3%), SEGAs in 9 (29.0%) and radial migration lines (RMLs) in 8 (25.8%). Overall, the brain lesion burden (RSS) was higher in individuals with TSC2 mutations compared with the others (p=0.002).
General and clinical/radiological features of the TSC group are illustrated in Table 1, whereas the genotype-phenotype correlations are shown in Table 2.

Biomarker levels in TSC
The plasma concentration of NfL was significantly higher in the whole TSC population than in the control group (6.31±5.32 vs 4.04±1.74 pg/mL, p=0.042; Figure 1); however, a much more striking difference was documented when assessing GFAP levels in TSC patients compared with HC (206.03±233.23 vs 53.08±32.88 pg/mL, p<0.001; Figure 2). No other biomarker was significantly increased, apart from a trend observed for Aβ40 (251.46±71.24 vs 217.95±53.32 pg/mL, p=0.057) and Aβ42 (13.33±3.53 vs 11.42±2.68 pg/mL, p=0.055). As expected, NfL was strongly related to age (ρ=0.717, p<0.001), which GFAP was not. When stratifying patients according to their genotype, we found that GFAP levels were significantly increased in both the TSC1 (p=0.029) and TSC2 (p<0.001) groups compared with HC, whereas only TSC2 patients - but not TSC1 ones - showed a higher NfL concentration than HC (p=0.039). Finally, tTau was significantly increased only in the TSC2 group (TSC2: 7.335±5.433 vs HC: 5.162±2.469 pg/mL, p=0.043), whereas for pTau181 a significant increase was observed in the TSC1 group alone (TSC1: 1.468±0.950 vs HC: 0.978±0.538 pg/mL, p=0.05). Complete data are shown in Table 3.

Correlations between biomarker levels and clinical-radiological features
Our analysis revealed that a history of ES was strongly associated with higher GFAP plasma levels (p<0.0001), whereas only a slight trend was observed in subjects with Lennox-Gastaut syndrome (p=0.07). However, no difference was found in GFAP concentration when comparing epileptic with non-epileptic patients, nor when stratifying the former according to their responsiveness to ASMs. Conversely, patients with epilepsy showed a significant increase in tTau concentration when compared with the others (8.08±5.34 vs 3.71±1.15 pg/mL, p=0.001).

GFAP appeared clearly related to the TAND spectrum (p<0.040). When stratifying the study population according to the degree of cognitive impairment (no/mild/moderate-severe ID), we found GFAP to be higher in all the sub-groups in comparison with HC (p<0.001). In addition, a significant difference was observed between individuals with moderate-severe ID and those with no ID (p=0.040, Figure 3). Aβ40 and Aβ42 plasmatic levels were increased only in patients with severe ID when compared with HC (p=0.025 and p=0.046, respectively), and a similar trend was observed for tTau as well (p=0.057).

As to the radiological findings, we found only GFAP levels to be strongly associated with the presence of SENs (p=0.009) and with a cortical tuber load greater than 3 (p<0.0001).

Finally, we calculated the NSS (mean: 3±1.80 for TSC1 and 4.68±2.87 for TSC2, p=0.278) and the RSS (mean: 2.77±1.20 for TSC1 and 3.31±1.10 for TSC2, p=0.253) to express the disease severity and to explore its possible relation with the plasma biomarkers. Indeed, we found that GFAP levels correlated with both NSS (ρ=0.498, p=0.005) and RSS (ρ=0.417, p=0.001), as illustrated in Figure 4. A less strong correlation was observed between tTau and NSS (ρ=0.388, p=0.034) but not with RSS. GFAP and tTau also appeared correlated themselves (ρ=0.391, p=0.033) (all the correlations are shown in the Supplementary Figure and Supplementary Table 2).

Discussion

In this study we found a significant increase of GFAP plasma levels in adult TSC patients regardless of their genotype, which was not the case with the other assessed biomarkers. GFAP concentration stayed significantly higher in all the analysed patient subgroups (i.e. epilepsy/no epilepsy, seizure-free/drug-resistant epilepsy, ES/no ES, severe-moderate/mild/no ID) when compared with HC. However, it clearly correlated with the disease clinical severity (expressed as the NSS); in addition, a strong association was documented with ES, severe ID and ASD. A slightly less strong but still significant correlation was also observed with the burden of the brain lesions, especially cortical-subcortical tubers and SENs.

GFAP is among the main constituents of the astrocyte intermediate filaments, and is physiologically involved in cell structure, mobility and proliferation, vesicular trafficking, neuron-glia interactions and blood-brain barrier (BBB) integrity[19]. A raise in GFAP levels is generally considered as a hallmark of reactive astrogliosis, defined as a spectrum of molecular, cellular and functional changes in astrocytes and interpreted as the astrocytic response to different central nervous system (CNS) injuries, whose protective or detrimental effect is still under debate[20]. Therefore, GFAP has been lately proposed as a potential biomarker in several contexts, ranging from acute conditions (e.g. traumatic brain injury - TBI)[21] to chronic diseases of both neuroinflammatory (e.g. multiple sclerosis and neuromyelitis optica spectrum disorder)[22,23] and neurodegenerative nature (e.g. Alzheimer's disease (AD) and FTD)[24-27]. For the first time, our study investigated GFAP in TSC, revealing a dramatic increase of its plasma concentration in adult patients. This finding is in line with the consistent evidence of prominent astrogliosis within cortical tubers (where - in fact - increased GFAP staining has been documented)[10,28], which could represent a primary cell-autonomous effect of the mTOR pathway hyperactivation directly within the astrocytes[11]. Considering the crucial role of this cell type in CNS development, synaptic regulation and energetic substrate supply, alterations in their morphology and function – which in TSC are 'embedded' in the patients' genes and likely start prenatally - could well result in the disruption of widespread neuronal networks, contributing to the development of epilepsy, behavioural disorders and cognitive impairment.

Our study also documented a correlation between GFAP concentration and the overall disease severity, as far as both clinical (NSS) and radiological (RSS) features were concerned: in this respect, it is reasonable to hypothesize that different levels of GFAP could reflect various degrees of astrogliosis, possibly contributing to the intrinsic variability of TSC neurological manifestations. Pathophysiological implications aside, this correlation is of particular clinical interest: indeed, were it confirmed on larger populations including children, GFAP could become a good candidate prognostic biomarker, and might also be useful to stratify patients for both clinical and research purposes (e.g. in clinical trials on therapeutic interventions).

Interestingly, although no difference was documented in GFAP levels between epileptic and non-epileptic individuals, nor between drug-responsive and drug-resistant ones, we found that GFAP was significantly higher in patients with a history of ES compared with the others. Since ES were also associated with severe ID and TSC2 mutation, this observation could be simply explained with spasms being part of an extremely severe phenotype. However, it is well known that recurrent seizures *per se* are able to cause a neuroinflammatory response[29], and some authors have hypothesized that in the immature brain inflammation may actually contribute to the progression of genetically determined cerebral lesions[30]. Therefore, we cannot exclude that ES might represent a sort of "second hit" phenomenon, exerting a time-dependent detrimental effect on cognitive and behavioral development, not to mention epileptogenesis.

As to the other assessed biomarkers, our study did not completely confirm the findings from a recent work documenting a raise of pTau concentration in the CSF of a small TSC cohort[6]: in fact, we found that the plasma levels of neither tTau nor pTau181 were higher in the whole TSC population *versus* HC, although pTau181 was significantly increased in the TSC1 group compared with controls, whereas tTau was higher in TSC2 patients alone. The latter also showed a significant increase in epileptic individuals compared with the others, and a trend in cases with severe ID. The mTOR pathway plays a well-known role in Tau synthesis and folding, so that its dysfunction could reasonably result in Tau intracellular accumulation and a consequent compromission of the cytoskeletal stability[31]. Taking into account both our observations and literature data, we might hypothesize that different mechanisms – ranging from neurodegenerative to neuroinflammatory processes (as suggested by histological findings)[32,33] – are involved to various (and variable) extents in TSC, possibly in relation to both genetic and acquired factors, which could partly justify the heterogeneity of its neurological manifestations.

This study has some limitations, first the small sample size, that might have affected the significance of some analyses. The biomarkers were assessed in blood instead of CSF, which is generally considered as a better surrogate of CNS pathology: however, it has been demonstrated that plasma GFAP is as good a biomarker as CSF GFAP (if not superior) in other contexts, such as AD[34]; moreover, the use of SIMOA, a widely validated ultra-sensitive assay, guarantees the reliability of the results[35,36]. In addition, considering that blood sample collection is far less invasive and easier to perform than a lumbar puncture, the use of plasma samples would potentially allow the longitudinal evaluation of GFAP levels and its correlation with disease progression. Finally, as to the kinetics of GFAP, it has been demonstrated that its plasma concentration raises within few hours from TBI (and seizures as well), peaks within the first day and slowly declines over days/weeks (although data are not consistent)[37,38]. Based on this premise, we collected blood samples after a ≥ 24-h seizure-free period, and although we cannot rule out some influence of recurring seizures on the detected GFAP levels, we think this rather unlikely, considering that no significant difference was documented between epileptic and non-epileptic patients, or within the epilepsy group when stratifying according to seizure frequency and responsiveness to ASMs.
In conclusion, our study documented a significant increase of GFAP plasma concentration in TSC adult patients, which appeared to correlate with the severity of their neurological phenotype. These findings support the central role of astrocytopathy - secondary to mTOR dysfunction - in determining TSC neurological manifestations, in line with the hypothesis that astrocytes could be both the target and the source of neuroinflammation and neurodegeneration in this condition[39]. Most importantly, our work suggests that plasma GFAP is a candidate prognostic biomarker in TSC: longitudinal studies on larger populations including pediatric patients are mandatory to investigate its use in both clinical practice and research to help predict disease severity, monitor its progression and assess the response to currently available disease-modifying therapies.

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Figure captions and legends

Figure 1. NfL plasmatic level

Abbreviations: HC: Healthy controls; NfL; Neurofilament Light Chain; NMI: patients with no mutations identified; TSC: Tuberous Sclerosis Complex. * $P \leq 0.05$, ** $P \leq 0.01$.

Figure 2. GFAP plasmatic level

Abbreviations: GFAP: Glial Fibrillary Acid Protein; HC: Healthy controls; NMI: patients with no mutations identified; TSC: Tuberous Sclerosis Complex. ** $P \leq 0.01$, *** $P \leq 0.001$; $^{++}$ $p \leq 0.01$.

Figure 3. GFAP plasmatic level in TSC patients stratified by the main neuropsychiatric features

Panel A: GFAP plasma level is higher in all patient subgroups compared with HC, but it does not significantly differ in epileptic vs non-epileptic patients, nor according to the responsiveness to antiseizure medications; Panel B: GFAP level is significantly higher in TSC patients with a history of epileptic spasms compared with the others; Panel C: in patients with moderate-severe ID, GFAP is significantly increased

than in those without ID (who have higher levels than HC); Panel D: ASD is associated with significantly higher plasma concentration of GFAP. Abbreviations: GFAP: Glial Fibrillary Acid Protein; HC: Healthy controls; NMI: patients with no mutations identified; TSC: Tuberous Sclerosis Complex. * $P \leq 0.05$, ** $P \leq 0.01$ *** $P \leq 0.001$.

Figure 4. Correlation between GFAP and neurological and radiological severity of TSC patients
The Severity score is the sum of the Neurological Severity Score (NSS) and the Radiological Severity Score (RSS). Abbreviations: GPFA: Glial Fibrillary Acid Protein

Table 1. Demographics, clinical features and radiological findings in TSC patients.

	TSC patients	%
Median age, years (IQR)	30 (24-47)	
Female	20	64.5
Genetics		
TSC1	9	29.0
TSC2	19	61.3
NMI	3	9.7
De novo mutations	20	64.5
Median age at diagnosis, months (IQR)	4 (0-36)	
Extraneurological involvemente		
- **Skin lesions**	22	71.0
- **Kidney**	17	54.8
Angiomyolipoma	5	29.4
Isolated cysts	3	17.6
Angiomyolipoma and cysts	9	53.0
- **Lung**	10	32.3
Lymphangioleiomyomatosis	4	40.0
MMPH	6	60.0
- **Cardiac rhabdomyoma**	10	32.3
- **Retinal phakomas**	4	12.9
- **Hamartomas in other regions (pancreas, liver)**	4	12.9
Neuropsychiatric manifestations		
- **Epilepsy**	23	74.2
Seizure free	10	43.5
Drug-resistant – LGS	11 – 2	47.8 – 8.7
History of epileptic spasms	14	60.9
- **TAND spectrum**	22	71.0
Anxiety	14	45.2
Depressive disorder	6	19.4
Impulsivity/aggression	12	38.7
OCD spectrum	8	25.8
ASD	11	35.5
ADHD	1	3.2
- **Cognitive impairment**	21	32.3
Mild	10	47.6
Moderate-severe	11	52.4
Neuroimaging findings		
- **Cortical tubers**	29	93.5
- **Tubers ≥ 3**	24	82.8
- **Subependymal nodules**	19	61.3
- **Radial migration lines**	8	11.3
- **SEGA**	9	29.0

Abbreviations: ADHD: attention deficit-hyperactivity disorder; ASD: autism spectrum disorder; IQR: interquartile range; LGS: Lennox-Gastaut syndrome; MMPH: multifocal micronodular pneumocyte hyperplasia; NMI: no mutations identified; OCD: obsessive-compulsive disorder; SEGA: subependymal giant cell astrocytoma; TSC: Tuberous Sclerosis Complex

Table 2. Genotype-phenotype correlations

	TSC1 (n=9)	TSC2 (n=19)	NMI (n=3)	p-value*	TSC2 vs all p-value#
Age, mean ± SD (y)	37.11±14.26	35.63±14.46	26.67±17.67	0.126	0.836
Gender	4 M, 5 F	7 M, 12 F	0 M, 3 F	0.371	0.842
Epilepsy, n (%)	7 (77.8)	15 (78.9)	1 (33.3)	0.235	0.676
LGS	0 (0.0)	2 (13.3)	0 (0.0)	0.558	0.526
Epileptic spasms	2 (22.2)	11 (57.9)	0 (0.0)	0.061	**0.032**
Psychiatric disorders, n (%)	5 (55.6)	16 (84.2)	1 (33.3)	0.095	0.056
ASD	1 (11.1)	10 (52.6)	0 (0.0)	**0.040**	**0.020**
ID, n (%)	7 (77.8)	13 (68.4)	1 (33.3)	0.360	1.000
Severe ID	1 (11.1)	10 (52.6)	0 (0.0)	**0.040**	**0.012**
Cortical tubers, n (%)	8 (88.9)	19 (100.0)	2 (66.7)	0.073	**<0.001**
Tubers ≥ 3	5 (62.5)	19 (100.0)	0 (0.0)	**<0.001**	**<0.001**
SENs, n (%)	4 (44.4)	15 (78.0)	0 (0.0)	**0.016**	**0.022**
RMLs, n (%)	2 (22.2)	6 (31.6)	0 (0.0)	0.488	0.433
SEGA, n (%)	4 (44.4)	5 (26.3)	0 (0.0)	0.311	0.704
RSS, mean ± SD	2.78±1.20	3.32±1.11	0.67±0.58	**0.009**	**0.039**
NSS, mean ± SD	3.00±1.80	4.68±2.87	1.00±1.00	0.058	**0.048**

The table compares TSC patients according to their genotype. Significant differences are indicated in bold. Abbreviations: ASD: autism spectrum disorder; ID: intellectual disability; LGS: Lennox-Gastaut syndrome; NSS: neurological severity score; RSS: radiological severity score; SD: standard deviation; SENs: subependymal nodules; TSC: Tuberous Sclerosis Complex

Table 3. Plasmatic biomarkers profile in TSC population vs HC

	HC	TSC1	TSC2	NMI
Aβ40	271.950±53.325	246.812±86.855	298.846±52.963	232.952±63.739*****
Aβ42	11.422±2.682	13.424±4.099	12.892±3.402	15.936±2.545******
tTau	5.162±2.469	7.141±4.708	7.335±5.433*****	3.734±0.181
pTau181	0.978±0.538	1.468±0.950*****	1.183±0.684	0.716±0.163
NfL	4.042±1.742	4.889±2.114	7.208±6.410******	4.407±2.083
GFAP	53.080±32.880	100.840±54.656******	271.799±271.778*******	70.016±20.940

*P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001 (vs HC); $$ p ≤ 0.01 (vs TSC2); ^^p ≤ 0.01 (vs NMI).

The table shows biomarker levels in HC and TSC patients stratified by genotype. Values are expressed in mean [pg/mL] ±SD. Abbreviations: AB: amyloid beta; GFAP: Glial Fibrillary Acid Protein; HC: Healthy controls; NfL: Neurofilament Light Chain; NMI: patients with no mutations identified; pTau: phosphorylated Tau; TSC: Tuberous Sclerosis Complex; tTau: total Tau