

## RESEARCH ARTICLE OPEN ACCESS

# [<sup>18</sup>F]Fluorodeprenyl-D2 PET as a Tool to Monitor Disease Activity in GAD65-Ab Autoimmune Encephalitis

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## ABSTRACT

**Objective:** To evaluate [<sup>18</sup>F]fluorodeprenyl-D2 ([<sup>18</sup>F]F-DED) positron-emission tomography (PET) imaging as a biomarker of disease activity in autoimmune encephalitis (AIE) associated with glutamic acid decarboxylase 65 (GAD65) antibodies.

**Methods:** [<sup>18</sup>F]F-DED PET was performed in 25 GAD65-AIE patients and 8 controls using dynamic (0–60 min) and static (30–60 min) acquisitions. Global astrogliosis was assessed by volumes of distribution (VT; 1-tissue-compartment model with carotid input) and standardized uptake values (SUV). Regional cerebellar and mesiotemporal (MT) uptake was normalized to global uptake (SUVr). PET measures were correlated with clinical phenotypes, MRI findings, and serum biomarkers (neurofilament light chain [sNfL], GAD65-Ab titers, and glial fibrillary acidic protein [sGFAP]).

**Results:** Clinical phenotypes included limbic encephalitis/temporal lobe epilepsy (LE/TLE; *n* = 17), stiff-person syndrome (*n* = 4), and cerebellar ataxia (CA; *n* = 4), with overlap in nine patients. Global [<sup>18</sup>F]F-DED uptake was higher in patients than controls. VT analysis showed increased cortical and MT uptake, while SUVr analysis demonstrated elevated MT uptake across the cohort. LE/TLE patients exhibited increased MT uptake, and CA patients showed higher cerebellar uptake. [<sup>18</sup>F]F-DED uptake did not correlate with sNfL or GAD65-Ab titers, but sGFAP showed associations with cerebellar and white-matter uptake. Regional uptake correlated with clinical severity in LE/TLE (MT lobe) and CA (cerebellar white matter).

**Interpretation:** [<sup>18</sup>F]F-DED PET reveals region-specific astrogliosis corresponding to clinical manifestations and disease severity in GAD65-AIE, supporting its potential as a monitoring tool for disease activity.

**Abbreviations:** [<sup>18</sup>F]F-DED, [<sup>18</sup>F]fluorodeprenyl-D2; Abs, antibodies; AIE, autoimmune encephalitis; CA, cerebellar ataxia; GAD65, glutamic acid decarboxylase 65; LE, limbic encephalitis; MAO-B, monoamine oxidase B; MT, mesiotemporal; PET, positron-emission-tomography; sGFAP, serum glial fibrillary acidic protein; sNfL, serum neurofilament light chain; SPS, stiff person syndrome; SUV, Standardized uptake values; TLE, temporal lobe epilepsy; VT, volumes of distribution.

Matthias Brendel and Franziska S. Thaler contributed equally to this study.

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## 1 | Introduction

Autoimmune encephalitis (AIE) associated with autoantibodies against the intracellular enzyme glutamic acid decarboxylase 65 (GAD65) is among the most common AIE subtypes. GAD65-AIE comprises a spectrum of clinical phenotypes, including limbic encephalitis (LE)/temporal lobe epilepsy (TLE), cerebellar ataxia (CA), stiff-person syndrome (SPS), and overlapping presentations [1, 2]. Disease onset may be subacute or insidious, and responses to immunotherapy are often incomplete [3]. Consequently, many patients develop a chronic disease course with persistent deficits and fluctuating activity, underscoring the need for reliable tools to monitor disease activity and treatment response.

Currently available paraclinical markers are limited. Magnetic resonance imaging (MRI) frequently shows mesiotemporal swelling or T2/fluid-attenuated inversion recovery (FLAIR) hyperintensities in GAD65-AIE [4, 5], but these abnormalities are often subtle, transient, and easily overlooked, particularly given the insidious onset of disease. As a result, MRI rarely provides a robust measure of ongoing disease activity. In patients with CA, cerebellar atrophy may become apparent in later stages [3]; however, this finding usually reflects advanced and largely irreversible tissue damage rather than active, potentially treatable inflammation.

Positron emission tomography (PET) has emerged as a complementary imaging modality in AIE. [ $^{18}\text{F}$ ]FDG PET has demonstrated high sensitivity for detecting characteristic metabolic patterns across multiple AIE subtypes [6] and is considered a valuable adjunct to MRI. Nevertheless, in GAD65-AIE, [ $^{18}\text{F}$ ]FDG PET appears to reveal fewer metabolic abnormalities than in other antibody-mediated encephalitides [7]. In recent years, novel PET tracers with different CNS targets have emerged. Translocator protein (TSPO) PET, using the radioligand [ $^{18}\text{F}$ ]DPA-714, which assesses microglial activation, has shown higher detection rates than MRI in mixed AIE cohorts [8] and has demonstrated increased, often asymmetric uptake in mesial temporal regions of patients with GAD65 antibodies [9].

PET imaging with the radiotracer [ $^{18}\text{F}$ ]fluorodeprenyl-D2 ([ $^{18}\text{F}$ ]F-DED) is a novel technique for evaluating regional monoamine oxidase B (MAO-B) expression. Since MAO-B is predominantly located on the mitochondrial membranes of glial cells and up-regulated in reactive astrocytes [10], [ $^{18}\text{F}$ ]F-DED PET enables in vivo assessment of reactive astrogliosis [11], a feature commonly observed in neuroinflammatory and neurodegenerative conditions. Astrocytes as the major cellular source of an increased MAO-B-PET signal was previously confirmed [12]. A transgenic mouse model of Alzheimer's disease demonstrated that [ $^{18}\text{F}$ ]F-DED signal elevation preceded changes detected by TSPO PET and  $\beta$ -amyloid PET [11]. We recently demonstrated the utility of [ $^{18}\text{F}$ ]F-DED PET for monitoring regional astrogliosis in patients with LGI1-associated AIE [13], suggesting that [ $^{18}\text{F}$ ]F-DED PET may also be helpful for evaluating the regional extent of inflammation in other forms of AIE.

Based on these considerations, we conducted a cross-sectional [ $^{18}\text{F}$ ]F-DED PET study comparing patients with GAD65-AIE to a control cohort. In addition, we examined associations between

tracer uptake, clinical phenotype and severity, and serum biomarkers of neuroaxonal injury and astroglial activation, aiming to explore the value of [ $^{18}\text{F}$ ]F-DED PET as a marker of disease activity in GAD65-AIE.

## 2 | Methods

### 2.1 | Study Design and Cohorts

This cross-sectional, monocentric study included  $n=25$  subjects with GAD65-AIE, presenting with limbic encephalitis (LE)/temporal lobe epilepsy (TLE), cerebellar ataxia (CA), or stiff person syndrome (SPS). Patients were diagnosed according to proposed diagnostic criteria [3] and followed up by neurologists specialized in AIE treatment at the Institute of Clinical Neuroimmunology, LMU Hospital. Clinical data, including the Montreal Cognitive Assessment (MoCa), the modified Rankin Score (mRS), the Scale for the Assessment and Rating of Ataxia (SARA), and the Clinical Assessment Scale in Autoimmune Encephalitis (CASE), were collected within 1 month of PET imaging. Additionally, composite scores were used based on a shortened, GAD65-AIE-tailored version of the clinical assessment scale in autoimmune encephalitis (CASE) score [14], as follows: LE: *Mild*: no seizures or seizures stopped  $\geq 3$  months before PET imaging; no cognitive impairment or cognitive impairment only subjectively reported and not detectable by testing. *Moderate*: ongoing seizures  $< 3$  months before PET imaging or cognitive impairment detectable in testing. *Severe*: ongoing seizures  $< 3$  months before PET imaging and cognitive impairment detectable in testing. CA: *Mild*: cerebellar signs detectable on clinical examination but not perceived by patient. *Moderate*: clinically affected, no walking aid required. *Severe*: walking aid required. SPS: *Mild*: only signs detectable on examination, such as brisk reflexes or increase startle reaction, without daily activity interference. *Moderate*: clinically affected; no walking aid required. *Severe*: walking aid required, at least outside of buildings (Figure 1A and Table 1).

The control cohort included 8 individuals (4 females, 4 males). Diagnoses in the control cohort included subjective cognitive dysfunction ( $n=5$ ) that was not confirmed by testing, transient neuropsychiatric symptoms ( $n=1$ ), oligodendroglioma WHO  $^{\circ}\text{II}$  ( $n=1$ ), and epilepsy ( $n=1$ ).

### 2.2 | Standard Protocol Approvals, Registrations, and Patient Consents

All subjects provided written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee (IRB numbers: 21–0721, 22–0997) and the German radiation protection authorities (BfS; ZD 3–22,464/2023–042-G).

### 2.3 | Antibody Testing

GAD65-antibody testing in serum and cerebrospinal fluid (CSF) was performed by indirect immunofluorescence (IIFT) using transfected HEK cells (Institute of Clinical Chemistry,

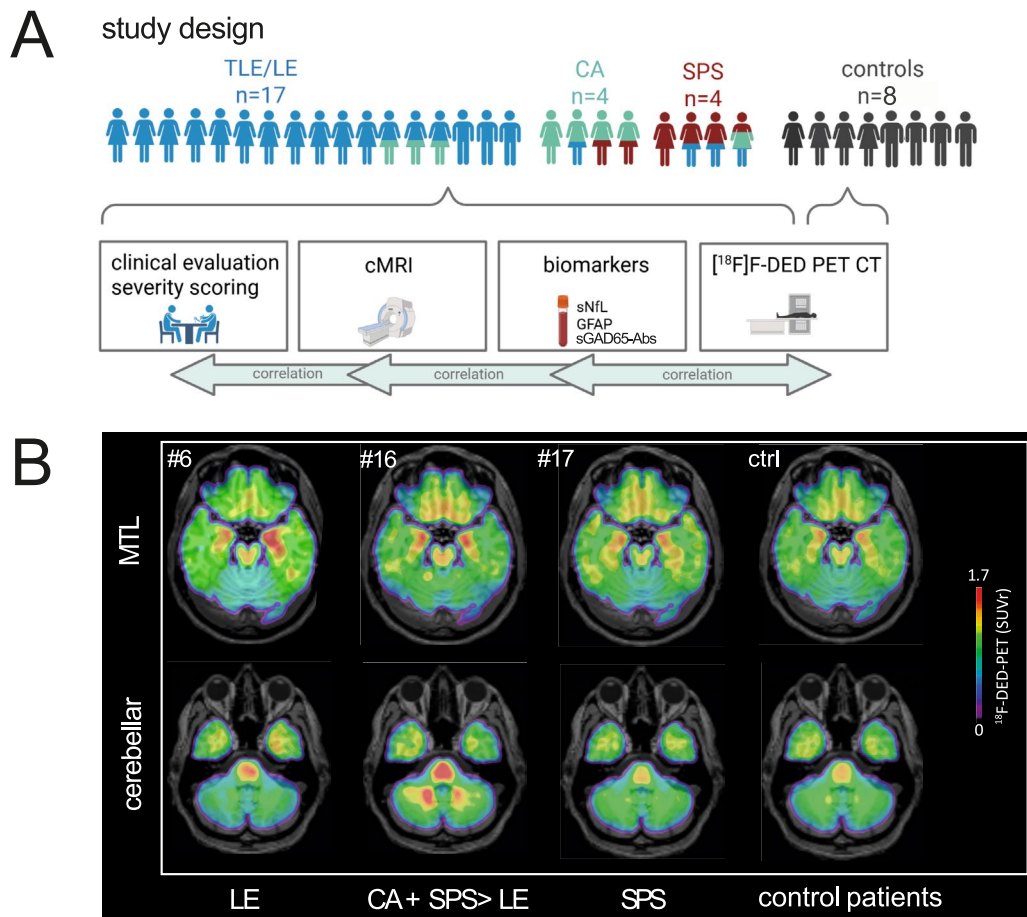


**FIGURE 1** | Study overview and qualitative assessment of regional  $[^{18}\text{F}]$ F-DED uptake. (A) Overview of study participants with different clinical phenotypes depicted in distinct colors: Limbic encephalitis (LE)/temporal lobe epilepsy (TLE): Blue; cerebellar ataxia (CA): Green; stiff person syndrome (SPS): Red, including overlapping clinical features with an overview of the analyzed parameter; generated in Biorender. (B)  $[^{18}\text{F}]$ F-DED uptake as visualized by SUVr with global mean scaling, is shown for three representative patients with GAD65-AIE: Patient #6 with LE/TLE, showing mesiotemporal  $[^{18}\text{F}]$ F-DED uptake; patient #16 with overlapping clinical features of CA, SPS, and LE, showing both mesiotemporal and cerebellar  $[^{18}\text{F}]$ F-DED uptake, and patient #17 with isolated SPS showing no increased  $[^{18}\text{F}]$ F-DED uptake. Additionally the average  $[^{18}\text{F}]$ F-DED uptake of eight controls is shown on the right.

EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany).

## 2.4 | sNfL and sGFAP Measurements

Serum sampling was performed within 6 months of PET imaging (except in patient #8 the interval was 28 months). Serum neurofilament light chain (sNfL) and serum glial fibrillary acidic protein (sGFAP) levels were quantified using the Simoa technology (Neurology 2-Plex B assay; sNfL, sGFAP) on the HX-analyzer as single measurements at the Institute of Clinical Chemistry, Universitätsklinikum Schleswig-Holstein, Campus Kiel.

## 2.5 | cMRI Evaluation

Clinical routine cMRI scans of the GAD65-AIE cohort were evaluated by a neuroradiologist blinded for the  $[^{18}\text{F}]$ F-DED PET results. MRI scans were acquired using different scanners but allowed standardized qualitative evaluation of: (I) Presence of T2/FLAIR hyperintensities in the mesiotemporal lobe (MTL),

(II) Presence of gadolinium enhancement in the MTL, (III) Volume of hippocampus, whole brain, MTL, and cerebellum. (Table 2).

## 2.6 | $[^{18}\text{F}]$ F-DED PET Imaging: Acquisition

$[^{18}\text{F}]$ F-DED PET was performed for diagnostic purposes in all GAD65-AIE patients between 06/2022 and 09/2025. Dynamic  $[^{18}\text{F}]$ F-DED PET scans (0–60 min post-injection) (mean: 166.9 MBq, SD: 23.0 MBq, range: 108.9 MBq) were performed at the Department of Nuclear Medicine, LMU University Hospital, as described previously [11]. Patients were asked to avoid nicotine or caffeine for 3 h prior to the PET scan, and active smokers were not imaged insofar as this was ascertainable from their medical history.

## 2.7 | $[^{18}\text{F}]$ F-DED PET Imaging: Analysis

Spatial normalization via structural MRI scans was achieved using PMOD. Volumes of distribution (VT) were determined

TABLE 1 | Overview of GAD65-AIE cohort at time of [<sup>18</sup>F]fluorodeprenyl-D2 PET imaging.

| Patient ID | Dominant phenotype | Overlapping phenotype | Disease duration at PET imaging (y) | Treatment at PET imaging <sup>a</sup> | all previous treatments                   | GAD65-Ab                                     |                                            |     |      |      | CASE score | LE severity <sup>c</sup> | CA severity <sup>c</sup> | SPS severity <sup>c</sup> |
|------------|--------------------|-----------------------|-------------------------------------|---------------------------------------|-------------------------------------------|----------------------------------------------|--------------------------------------------|-----|------|------|------------|--------------------------|--------------------------|---------------------------|
|            |                    |                       |                                     |                                       |                                           | GAD65-Ab titer (serum) at PET imaging (1: x) | GAD65-Ab titer (CSF) at PET imaging (1: x) | mRs | SARA | MOCA |            |                          |                          |                           |
| #1         | LE                 | /                     | 12                                  | MMF                                   | GCs, PLEX, ivIG, Aza, MMF, MTX, rituximab | 1600                                         | 50                                         | 2   | 0    | 26   | 6          | 3                        | 0                        | 0                         |
| #2         | LE                 | /                     | 1                                   | none                                  | none                                      | 25,600                                       | 200                                        | 1   | 0    | nd   | 1          | 1                        | 0                        | 0                         |
| #3         | LE                 | /                     | 5                                   | none                                  | GCs, PLEX, rituximab                      | 6400                                         | 50                                         | 2   | 0    | 30   | 3          | 3                        | 0                        | 0                         |
| #4         | SPS                | LE                    | 13                                  | ivIG, MMF                             | GCs, ivIG, MMF, MTX                       | 2560                                         | nd                                         | 1   | 1    | 30   | 1          | 1                        | 0                        | 2                         |
| #5         | LE                 | /                     | 8                                   | none                                  | GCs, PLEX, rituximab                      | 1600                                         | 100                                        | 1   | 0    | 30   | 2          | 2                        | 0                        | 0                         |
| #6         | LE                 | CA                    | 5                                   | none                                  | GCs, rituximab                            | 3200                                         | 100                                        | 2   | 1    | 27   | 3          | 2                        | 1                        | 0                         |
| #7         | CA                 | SPS                   | 2                                   | rituximab                             | GCs, PLEX, ivIG, rituximab                | 10                                           | 10                                         | 3   | 16   | nd   | 2          | 0                        | 3                        | 1                         |
| #8         | LE                 | /                     | 8                                   | none                                  | GCs, PLEX, ivIG, Aza, rituximab           | 400                                          | 50                                         | 2   | 1    | 29   | 2          | 3                        | 0                        | 0                         |
| #9         | LE                 | /                     | 9                                   | GCs, PLEX                             | GCs, PLEX                                 | 3200                                         | 100                                        | 1   | 0    | 28   | 4          | 3                        | 0                        | 0                         |
| #10        | LE                 | CA                    | 9                                   | rituximab                             | GCs, ivIG, rituximab                      | 800                                          | 50                                         | 2   | nd   | 26   | 4          | 3                        | 2                        | 0                         |
| #11        | LE                 | /                     | 4                                   | none                                  | GCs                                       | 400                                          | 100                                        | 2   | nd   | nd   | 2          | 2                        | 0                        | 0                         |
| #12        | LE                 | /                     | 3                                   | GCs, PLEX                             | GCs, PLEX                                 | 12,800                                       | 100                                        | 1   | 0    | 29   | 2          | 2                        | 0                        | 0                         |
| #13        | CA                 | SPS                   | 12                                  | PLEX, MTX                             | GCs, PLEX, MTX, rituximab                 | 6400                                         | nd                                         | 3   | 12.5 | 27   | 2          | 0                        | 3                        | 1                         |

(Continues)

TABLE 1 | (Continued)

| Patient ID | Dominant phenotype | Overlapping phenotype | Disease duration at PET imaging (y) | Treatment at PET imaging <sup>a</sup> | GAD65-Ab                              |                                              |                                            |     |      | CASE score | LE severity <sup>c</sup> | CA severity <sup>c</sup> | SPS severity <sup>c</sup> |
|------------|--------------------|-----------------------|-------------------------------------|---------------------------------------|---------------------------------------|----------------------------------------------|--------------------------------------------|-----|------|------------|--------------------------|--------------------------|---------------------------|
|            |                    |                       |                                     |                                       | Treatment at PET imaging <sup>a</sup> | GAD65-Ab titer (serum) at PET imaging (1: x) | GAD65-Ab titer (CSF) at PET imaging (1: x) | mRs | SARA |            |                          |                          |                           |
| #14        | CA                 | LE                    | 20                                  | none                                  | GCs                                   | 3200                                         | nd                                         | 2   | 8.5  | 22         | 2                        | 2                        | 0                         |
| #15        | LE                 | /                     | 3                                   | GCs                                   | GCs                                   | 25,600                                       | nd                                         | 1   | 1    | 25         | 4                        | 2                        | 0                         |
| #16        | SPS                | CA, LE                | 9                                   | ivIG                                  | GCs, ivIG, Aza                        | 25,600                                       | 1600                                       | 2   | 3.5  | 28         | 4                        | 1                        | 3                         |
| #17        | SPS                | /                     | 1                                   | ivIG, ustekinumab <sup>b</sup>        | ivIG, ustekinumab <sup>b</sup>        | 1600                                         | nd                                         | 3   | nd   | 27         | 2                        | 0                        | 3                         |
| #18        | LE                 | CA                    | 1                                   | none                                  | GCs, Aza                              | 12,800                                       | 100                                        | 2   | 7    | 9          | 4                        | 2                        | 0                         |
| #19        | CA                 | /                     | 3                                   | Aza                                   | GCs, PLEX, Aza                        | 6400                                         | 100                                        | 1   | 3.5  | nd         | 1                        | 0                        | 0                         |
| #20        | LE                 | /                     | 0                                   | none                                  | GCs                                   | 25,600                                       | 100                                        | 0   | 0    | 30         | 1                        | 1                        | 0                         |
| #21        | SPS                | LE                    | 0                                   | ivIG                                  | ivIG                                  | 25,600                                       | nd                                         | 1   | 0    | 30         | 4                        | 2                        | 2                         |
| #22        | LE                 | /                     | 1                                   | GCs                                   | GCs                                   | 6400                                         | 100                                        | 0   | 0    | 28         | 1                        | 1                        | 0                         |
| #23        | LE                 | /                     | 6                                   | none                                  | none                                  | 3200                                         | 10                                         | 1   | 0    | 29         | 2                        | 2                        | 0                         |
| #24        | LE                 | /                     | 0                                   | none                                  | none                                  | 6400                                         | 10                                         | 0   | 0    | 29         | 1                        | 1                        | 0                         |
| #25        | LE                 | /                     | 5                                   | none                                  | none                                  | 12,800                                       | 100                                        | 1   | 0    | 24         | 4                        | 3                        | 0                         |
| median     |                    |                       | 5                                   |                                       |                                       | 6400                                         | 100                                        | 1.0 | 0    | 28         | 2.0                      | 1.5                      | 0                         |
| IQR        |                    |                       | 6.8                                 |                                       |                                       | 11,200                                       | 50                                         | 1.0 | 2.25 | 3          | 2.0                      | 1                        | 0                         |

Abbreviations: Aza, azathioprine; CA, cerebellar ataxia; CASE, Clinical Assessment Scale in Autoimmune Encephalitis; GCs, glucocorticosteroids; IQR, interquartile range; ivIG, monthly intravenous immunoglobulins; LE/TLE, limbic encephalitis/temporal lobe epilepsy; m, male; MMF, mycophenolate-mofetil; MOCA, Montreal cognitive assessment scale; mRs, modified Rankin scale; MTX, methotrexate; nd, no data; PLEX, plasma exchange; SARA, scale for assessment and rating of ataxia; SPS, stiff person syndrome.

<sup>a</sup>Within 3 months prior.

<sup>b</sup>Due to comorbidities.

<sup>c</sup>Mild: 1, moderate: 2, severe: 3.

TABLE 2 | MRI characteristics of patient cohort.

| Patient ID | Dominant phenotype | Overlapping phenotype | Disease duration at MRI imaging (y) | Interval between MRI and PET imaging (m) |      | Field strength | 3D FLAIR | T2/FLAIR hyperintensity |     | Gd-enhancement |    | Hippocampal volume |    | Whole brain volume |   | MTL volume |   | Cerebellar volume |   |
|------------|--------------------|-----------------------|-------------------------------------|------------------------------------------|------|----------------|----------|-------------------------|-----|----------------|----|--------------------|----|--------------------|---|------------|---|-------------------|---|
|            |                    |                       |                                     |                                          |      |                |          |                         |     |                |    |                    |    |                    |   |            |   |                   |   |
| #1         | LE                 | /                     | 12                                  | 0                                        | 3T   | yes            |          | ++                      | +   | no             | no | →                  | →  | →                  | → | ↑          | ↓ | →                 | → |
| #2         | LE                 | /                     | 1                                   | 6                                        | 1.5T | no             |          | +                       | ++  | nd             | nd | →                  | →  | →                  | → | →          | → | →                 | → |
| #3         | LE                 | /                     | 5                                   | 3                                        | 3T   | yes            |          | -                       | ++  | no             | no | →                  | →  | →                  | → | ↑          | → | →                 | → |
| #4         | SPS                | LE                    | 13                                  | 39 <sup>a</sup>                          | 1.5T | no             |          | -                       | -   | no             | no | →                  | ↓  | →                  | → | →          | → | →                 | → |
| #5         | LE                 | /                     | 8                                   | 3                                        | 3T   | no             |          | +                       | -   | no             | no | →                  | →  | →                  | → | →          | → | →                 | → |
| #6         | LE                 | CA                    | 5                                   | 0                                        | 1.5T | yes            |          | +                       | +   | no             | no | →                  | →  | ↓                  | → | ↑          | → | ↓                 | → |
| #7         | CA                 | SPS                   | 2                                   | 9                                        | 3T   | yes            |          | +                       | +   | nd             | nd | →                  | →  | →                  | → | →          | → | ↓                 | → |
| #8         | LE                 | /                     | 8                                   | 25                                       | 3T   | yes            |          | +                       | +++ | nd             | nd | →                  | ↓  | →                  | → | →          | → | →                 | → |
| #9         | LE                 | /                     | 9                                   | 3                                        | 3T   | yes            |          | +                       | ++  | no             | no | ↓                  | ↓  | →                  | → | →          | → | →                 | → |
| #10        | LE                 | CA                    | 9                                   | 4                                        | 1.5T | yes            |          | ++                      | ++  | nd             | nd | ↑                  | ↑  | →                  | → | ↑          | → | →                 | → |
| #11        | LE                 | /                     | 4                                   | 0                                        | 3T   | yes            |          | -                       | -   | no             | no | →                  | →  | →                  | → | →          | → | →                 | → |
| #12        | LE                 | /                     | 3                                   | 3                                        | 3T   | yes            |          | +                       | ++  | nd             | nd | ↑                  | ↑↑ | →                  | → | ↑          | → | →                 | → |
| #13        | CA                 | SPS                   | 12                                  | 0                                        | 3T   | yes            |          | -                       | -   | no             | no | ↓                  | ↓  | ↓                  | → | →          | → | ↓                 | → |
| #14        | CA                 | LE                    | 20                                  | 8                                        | 3T   | yes            |          | -                       | -   | no             | no | ↓                  | ↓  | →                  | → | ↑          | → | →                 | → |
| #15        | LE                 | /                     | 3                                   | 2                                        | 1.5T | no             |          | +                       | +   | nd             | nd | →                  | ↓  | ↓                  | → | ↑          | → | →                 | → |
| #16        | SPS                | CA, LE                | 9                                   | 2                                        | 1.5T | no             |          | +                       | +   | no             | no | →                  | →  | →                  | → | →          | → | ↓                 | → |
| #17        | SPS                | /                     | 1                                   | 2                                        | 1.5T | no             |          | +                       | +   | nd             | nd | →                  | ↓  | →                  | → | →          | → | ↓                 | → |
| #18        | LE                 | CA                    | 1                                   | 0                                        | 3T   | yes            |          | -                       | -   | nd             | nd | ↓↓                 | ↓↓ | ↓                  | → | ↓          | → | →                 | → |
| #19        | CA                 | /                     | 3                                   | 2                                        | 3T   | no             |          | -                       | -   | no             | no | →                  | →  | →                  | → | ↑          | → | →                 | → |
| #20        | LE                 | /                     | 10                                  | 3                                        | 3T   | no             |          | -                       | -   | nd             | nd | →                  | →  | ↓                  | → | →          | → | ↓                 | → |
| #21        | SPS                | LE                    | 0                                   | 3                                        | 3T   | yes            |          | +                       | +   | no             | no | →                  | →  | →                  | → | ↑↑         | → | →                 | → |
| #22        | LE                 | /                     | 1                                   | 7                                        | 3T   | yes            |          | +                       | -   | no             | no | →                  | →  | →                  | → | ↑          | → | →                 | → |
| #23        | LE                 | /                     | 7                                   | 0                                        | 3T   | yes            |          | +                       | +   | nd             | nd | →                  | →  | →                  | → | ↑          | → | →                 | → |
| #24        | LE                 | /                     | 0                                   | 3                                        | 3T   | yes            |          | +                       | -   | no             | no | →                  | →  | →                  | → | ↑          | → | →                 | → |

(Continues)

TABLE 2 | (Continued)

[illegible]

Abbreviations: +, minimal; ++, moderate; ++++, substantial; ↑, volume increase; ↓, volume decrease; ↑↑, swelling; ↓↓, atrophy; CA, cerebellar ataxia; Gd, gadolinium; LE/TLE, limbic encephalitis/temporal lobe epilepsy; m, months; MTL, mesiotemporal lobe; nd, not done; SPS, stiff person syndrome; y, years.

Due to cardiac pacemaker no repeat MRI imaging possible.

using a one-tissue-compartment model (1TC2k) with a carotid image-derived input function. For simplified quantification, standardized uptake values (SUV) were extracted from a late time window (30–60 min post-injection). In three patients with GAD65 AIE, only the late static scan was performed due to more severe disability and the need for reduced scanning time. Regions of interest—including the MTL and cerebellar white matter [15]—were manually defined in PMOD, whereas a pre-defined composite of cortical and subcortical regions served as a reference for global astrogliosis assessment. An exploratory PET-to-serum-biomarker correlation analysis was performed using a voxel-wise regression model (sNfL and sGFAP as predictors) in statistical parametric mapping (SPM) implemented in MATLAB (v2011-R2016).

## 2.8 | Statistical Analysis

GraphPadPrism (Version 10) was used for statistical analysis, and Biorender and Adobe Illustrator for figure creation.  $p < 0.05$  were considered statistically significant. Bonferroni correction was applied to control for multiple testing and  $p$ -values were adjusted accordingly. Normal distribution was tested using the Shapiro–Wilk test. For multi-comparison testing, groups were compared by ANOVA. Comparison of two groups was performed by unpaired  $t$ -test. Correlations were assessed using linear regression. Difference in sex ratio was compared by Fisher's exact test. A bootstrapping analysis with 1000 samples was performed to validate associations between blood biomarkers and F-DED PET imaging.

### 3 | Results

### 3.1 | Study Cohort

A total of 25 patients with GAD65-AIE were included in the study ( $n=17$  with LE/TLE,  $n=4$  with SPS,  $n=4$  with CA, with overlapping clinical phenotypes in nine individuals) (Figure 1A). As expected in GAD65-AIE, there was a strong female predominance ( $n=22$ ) which could not be replicated in the control cohort (significant difference, Fisher's exact test,  $p=0.04$ ). The median age at PET imaging was 48 years (IQR: 29 years), while the median age of the control cohort was 59.5 years (IQR: 6.5 years) (no significant difference, Mann-Whitney test,  $p=0.27$ ). The median disease duration was 5 years (IQR: 6.8 years). At the time of PET imaging, 12 patients were untreated, while 13 were receiving immunomodulatory or immunosuppressive treatment, as specified in Table 1. At time of diagnosis all patients revealed high-titer GAD65-Ab titers in serum (median: 1:6400) and CSF (median: 1:100) with evidence of intrathecal GAD65-Ab synthesis (Table 1). Information on anti-seizure medications and psychiatric co-mediations is provided in Table S1. Clinical severity was assessed using several standardized scales. The median mRS score was 1.0 (IQR: 1.0) for the overall cohort (LE/TLE: 1.0 [IQR: 1.0], CA: 2.5 [IQR: 1.25], SPS: 1.5 [IQR: 1.0]). The median SARA score was 0 (IQR: 2.25) for the overall cohort (LE/TLE: 0 [IQR: 1.0], CA: 10.5 [IQR: 6.125], SPS: 0 [IQR: 0]). The median MoCA score was 28 out of 30 (IQR: 3.0) for the overall cohort, suggesting minimal cognitive impairment in most patients (LE/TLE: 28.0 [IQR 2.25], CA: 24.5 [IQR: 2.5], SPS: 30 [IQR: 2.0]). The median CASE score was 2.0 (IQR:

2.0) for the overall cohort (LE/TLE: 2.0 [IQR 2.0], CA: 2.0 [IQR: 0.25], SPS: 2.5 [IQR: 2.0]). Additionally, severity ratings based on composite clinical scores (see Methods) were as follows: LE as main manifestation: *mild*:  $n=3$ , *moderate*:  $n=6$ , *severe*:  $n=5$ . LE as overlapping manifestation: *mild*:  $n=2$ , *moderate*:  $n=2$ . CA as main manifestation: *moderate*:  $n=2$ , *severe*:  $n=2$ . CA as overlapping manifestation: *mild*:  $n=1$ , *moderate*:  $n=3$ . SPS as main manifestation: *moderate*:  $n=2$ , *severe*:  $n=2$ . SPS as overlapping manifestation: *mild*:  $n=2$  (Table 1). Clinical scoring was performed within 1 month of PET acquisition.

### 3.2 | Qualitative Assessment of [ $^{18}\text{F}$ ]F-DED PET Imaging in GAD65-AIE

We first assessed whether [ $^{18}\text{F}$ ]F-DED uptake is increased in GAD65-AIE patients compared to controls, and whether regional uptake patterns correspond with clinical phenotypes. Visual analysis revealed distinct uptake patterns: in patients with LE/TLE we observed uptake in the MTL; in patients with CA we observed uptake in the cerebellar white matter; in patients with overlapping LE/TLE and CA we observed focal uptake in both areas; in patients with isolated SPS no increased [ $^{18}\text{F}$ ]F-DED uptake was observed (Figure 1B).

### 3.3 | Quantitative Assessment of [ $^{18}\text{F}$ ]F-DED PET by Region and Phenotype

We next quantified [ $^{18}\text{F}$ ]F-DED uptake in patients with GAD65-AIE and controls using dynamic VT values (ITC2k model with carotid-derived input function). Global VT signal was significantly higher in GAD65-AIE patients than in controls (Figure 2A). The global VT signal increase was most pronounced in the LE/TLE subgroup (Figure S1A). Furthermore, GAD65-AIE patients showed significantly increased [ $^{18}\text{F}$ ]F-DED uptake in the cerebral cortex (*adjusted  $p$  after Bonferroni-correction* = 0.027) as well as in the MTL (*adjusted  $p$  after Bonferroni-correction* = 0.009) (Figure 2B). To assess regional uptake differences independent of global uptake levels, we furthermore used SUV values normalized to global cortical and subcortical uptake (SUVr GLM) (Figure S1B). SUVr GLM values correlated with VTr GLM values ( $p < 0.0001$ ) (Figure S1C), suggesting that blood flow was not a confounder of static late-phase imaging. SUVr GLM analysis showed increased MT uptake in GAD65-AIE patients compared to controls (*adjusted  $p$  after Bonferroni-correction* = 0.012). No significant differences were observed in cortical and cerebellar uptake (Figure 2C). When comparing different clinical phenotypes (dominant clinical phenotype), we detected a significant increase of [ $^{18}\text{F}$ ]F-DED uptake in the MTL in patients with LE/TLE compared to controls (*adjusted  $p$  after Bonferroni-correction* = 0.0018), but not in patients with CA or SPS and in cortex or cerebellum (Figure 2D). When analyzing all patients with CA (including overlapping phenotypes), we observed increased cerebellar white matter uptake compared to non-CA patients ( $p = 0.02$ ) (Figure S2A), but not when comparing MT uptake in patients with LE (including overlapping phenotypes) compared to patients with non-LE. Notably, the number of patients without LE/TLE-phenotype was low ( $n = 4$ ) (Figure S2B). Comparison

of regional [ $^{18}\text{F}$ ]F-DED uptake between treated and untreated patients showed no significant differences (Figure S2C). No correlation was found between uptake in MT or cerebellar white matter regions and disease duration in LE/TLE or CA patients, respectively (Figure S2D,E).

### 3.4 | Longitudinal Assessment of [ $^{18}\text{F}$ ]F-DED PET Imaging

Next, we assessed changes of cortical, cerebellar, and MTL in four patients with available follow-up scans. In patient #7, presenting with cerebellar ataxia, the BL scan was performed 2 years and the FU scan 4 years after disease onset while continuing therapy with rituximab. Reduced cerebellar uptake at follow-up was observed matching to a SARA score decline from 16 (BL) to 12 (FU). In patient #15, presenting with LE/TLE, the BL scan was performed 3 years and the FU scan 4 years after disease onset while continuing therapy with oral corticosteroids. An increase of mesiotemporal tracer uptake at follow-up was observed matching to a re-emergence of seizure activity and an increased CASE score (BL: 3, FU: 4). In patient #21, presenting with SPS and overlapping LE/TLE, the BL scan was performed 7 months after disease onset and the FU scan 10 months later after treatment with high-dose corticosteroids and under treatment with mycophenolate-mofetil. A reduction of mesiotemporal tracer uptake was observed clinically associated with seizure cessation and decreased CASE-score (BL: 4, FU: 2). In patient #23, presenting with LE/TLE, the BL scan was performed 7 years after disease onset and the FU scan 3 months later after treatment with high-dose corticosteroids and plasma exchange. A reduction of mesiotemporal tracer uptake was observed clinically associated with a reduction of seizure frequency and intensity (Figure 3A,B).

### 3.5 | Correlation of [ $^{18}\text{F}$ ]F-DED Uptake With Biomarkers

Next, we evaluated the correlations between [ $^{18}\text{F}$ ]F-DED uptake and the serum levels of biomarkers potentially reflecting disease activity in patients with GAD65-AIE: serum neurofilament light chain (sNfL) as a marker for neuroaxonal damage, serum glial fibrillary acidic protein (sGFAP) as a marker for astroglial activation, and serum GAD65-Ab titers. No significant correlations were observed between sNfL levels and GAD65-Abs levels and regional [ $^{18}\text{F}$ ]F-DED uptake in cortex, MT structures, or cerebellum (Figures 4A and S3A), nor when applying an exploratory voxel-wise analysis (Figures 4C,D and S3B,C). For sGFAP, a correlation trend of cerebellar tracer uptake and sGFAP levels was observed, while no significant correlation with cortical and MT uptake was observed (Figure 4B). Furthermore, an exploratory voxel-wise analysis revealed an association of [ $^{18}\text{F}$ ]F-DED correlations with sGFAP levels with predominance in the subcortical white matter, basal ganglia, cerebellar white matter, and pons (Figure 4C,D). The association of sGFAP and [ $^{18}\text{F}$ ]F-DED PET was significant when a bootstrapping analysis with 1000 samples was performed ( $p = 0.034$ ), whereas GAD65 antibodies ( $p = 0.091$ ) and NfL ( $p = 0.786$ ) did not indicate a significant association with [ $^{18}\text{F}$ ]F-DED PET.

A

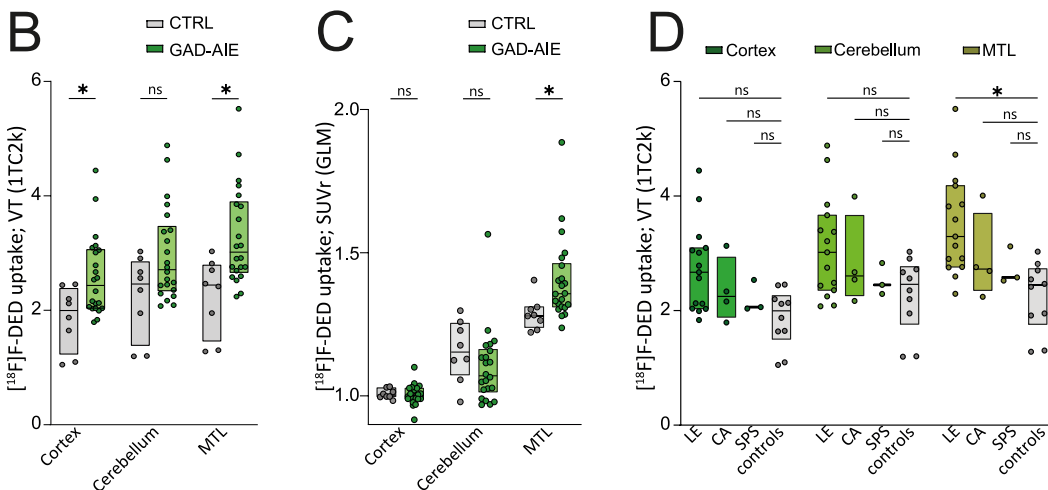
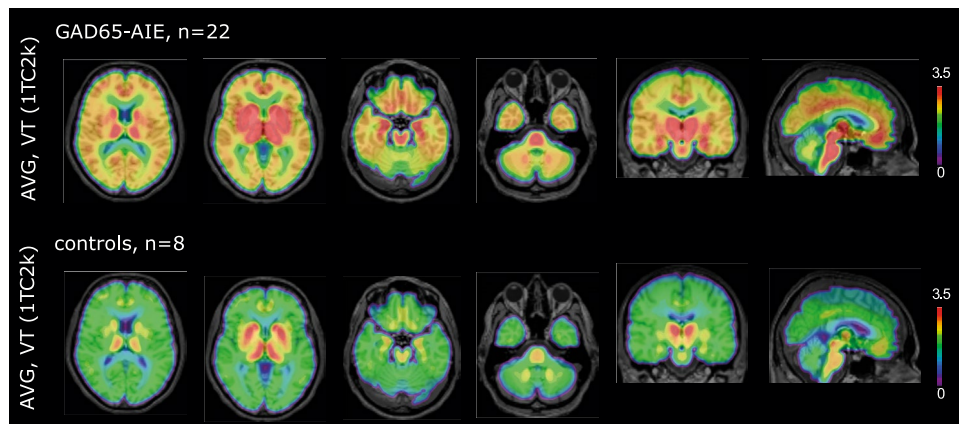


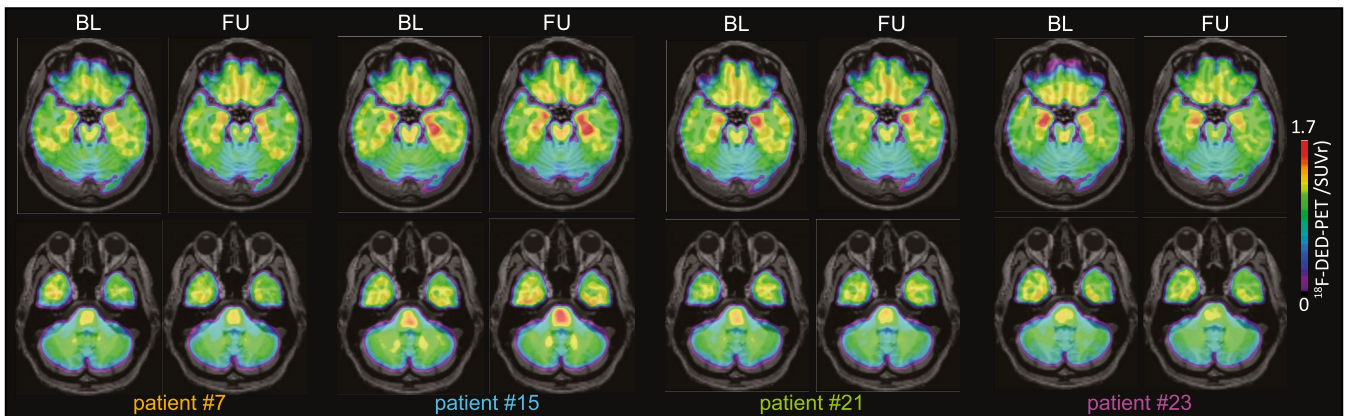
**FIGURE 2** | Quantitative assessment of  $[^{18}\text{F}]$ F-DED uptake. (A) Global  $[^{18}\text{F}]$ F-DED uptake, quantified by volumes of distribution (VT, 1TC2k model), is shown as an average for GAD65-AIE patients ( $n=22$ ) and controls ( $n=8$ ). VT values could not be calculated for 3 GAD65-AIE patients due to the absence of dynamic imaging. (B)  $[^{18}\text{F}]$ F-DED uptake was quantified by VT and compared between GAD65-AIE patients and controls in the cortex, mesiotemporal (MT) lobe (MTL), and cerebellar white matter. Unpaired  $t$ -test with Welch-correction were used to determine differences. Bonferroni correction was applied to control for multiple testing. (C)  $[^{18}\text{F}]$ F-DED uptake (standard uptake values [SUVr] with global mean scaling) was compared between GAD65-AIE patients and controls in the cortex, mesiotemporal (MT) lobe (MTL), and cerebellar white matter. Unpaired  $t$ -test with Welch-correction were used to determine differences. Bonferroni correction was applied to control for multiple testing. (D)  $[^{18}\text{F}]$ F-DED uptake was quantified by VT in the cortex, MTL, and cerebellar white matter and was compared across different clinical phenotypes (dominant clinical phenotype) and controls. Two-way ANOVA was used to determine differences. Bonferroni correction was applied to control for multiple testing.

### 3.6 | Correlation of $[^{18}\text{F}]$ F-DED Uptake With Clinical Severity and cMRI Findings

To evaluate concordance between clinical severity and astroglia PET imaging, we generated a heatmap of clinical and PET phenotypes and severity grades (Figure 5A). Except for one patient (#19), regional PET tracer uptake in the MTL or cerebellum corresponded with clinical presentation of LE/TLE or CA, respectively. Patient #19 had clinical CA with cerebellar tracer uptake, but also MT uptake. In this patient due to a language barrier, signs of LE may have been unnoticed. Notably,  $[^{18}\text{F}]$ F-DED PET detected MT abnormalities in 90.5% (19/21) of GAD65-LE/TLE patients (including patients with LE as dominant as well as overlapping phenotype). The two remaining GAD65-LE/TLE patients without MT tracer uptake reported only subtle LE symptoms (clinical severity grade: 1). In patients with GAD65-CA (including patients with CA as dominant as

well as overlapping phenotype) increased cerebellar tracer uptake was observed in 87.5% (7/8) patients. The one remaining GAD65-CA patient without cerebellar tracer uptake showed only subtle CA symptoms (clinical severity grade: 1). When visually analyzing the MRI patterns at the time of PET imaging, 76.2% (16/21) of patients with GAD65-LE/TLE (either as dominant or overlapping phenotypes) showed T2/FLAIR hyperintensities in the MTL. However, these were only minimal in 50% (8/16) of these patients. 52% (11/21) showed volume alterations of the hippocampus (either increased or decreased volume) and 62% (13/21) revealed volume alterations of the MTL. Cerebellar volume reduction was found in 50% (4/8) of patients presenting with GAD65-CA either as dominant or overlapping phenotype (Table 2). Next, we compared  $[^{18}\text{F}]$ F-DED PET signals with MRI findings. In patients with LE/TLE (as a dominant or overlapping phenotype) we found significantly higher MT  $[^{18}\text{F}]$ F-DED tracer uptake in patients with detectable MT FLAIR/T2

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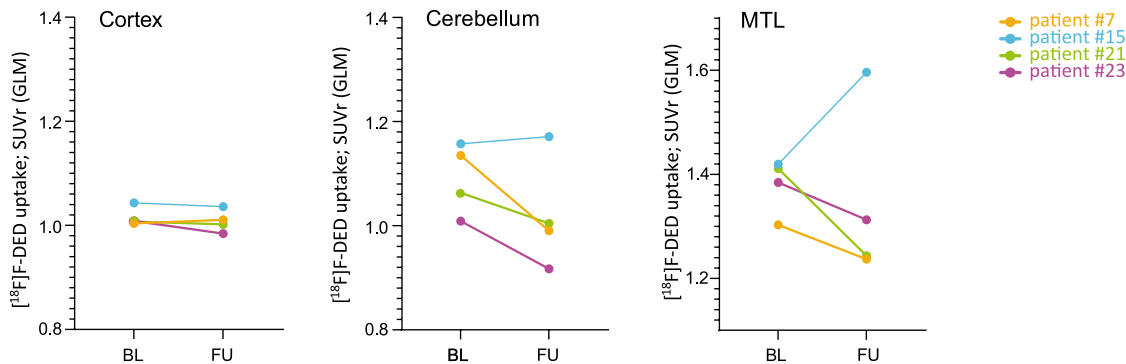


**FIGURE 3** | Longitudinal assessment of  $[^{18}\text{F}]\text{F-DED}$  uptake in four cases. (A) Baseline (BL) and follow-up (FU) scans of the patients are shown. In patient #7, presenting with cerebellar ataxia, the BL scan were performed 2years and the FU scan 4years after disease onset while continuing therapy with rituximab. In patient #15, presenting with LE/TLE, the BL scan were performed 3years and the FU scan 4years after disease onset while continuing therapy with oral corticosteroids. In patient #21, representing with SPS and overlapping LE/TLE, the BL scan was performed 7months after disease onset and the FU scan 10months later after treatment with high dose corticosteroids, and under treatment with mycophenolate-mofetil. In patient #23, presenting with LE/TLE, the BL scan was performed 7years after disease onset and the FU scan 3months later after treatment with high dose corticosteroids and plasma exchange. (B)  $[^{18}\text{F}]\text{F-DED}$  uptake (standard uptake values [SUVr] with global mean scaling) in cortex, mesio-temporal (MT) lobe (MTL), and cerebellar white matter is depicted at BL and FU for the individual patients.

hyperintensities than in patients without such MRI abnormalities ( $p=0.04$ ) (Figure S4A). No difference was observed when comparing cerebellar tracer uptake in CA patients with or without cerebellar volume decrease (Figure S4B).

Next, we assessed whether regional  $[^{18}\text{F}]\text{F-DED}$  uptake correlates with clinical severity. Representative  $[^{18}\text{F}]\text{F-DED}$  PET images illustrating the different grading levels of MT and cerebellar uptake are illustrated in Figure 5B. In LE/TLE patients, we observed a significant correlation between clinical severity (as quantified by LE-adjusted CASE-score grading from 0 to 3) and MTL  $[^{18}\text{F}]\text{F-DED}$  uptake as quantified by SUVr GLM ( $p=0.036$ ) (Figure 5C). Similarly, we observed a significant correlation of CASE scoring and MTL  $[^{18}\text{F}]\text{F-DED}$  uptake in LE/TLE patients ( $p=0.007$ ) (Figure 5D). Clinical severity in CA (CA-adjusted CASE-score grading from 0 to 3) correlated with cerebellar white matter uptake ( $p=0.014$ ) (Figure 5E). A trend toward correlation was also observed when we correlated cerebellar tracer uptake with the SARA score ( $p=0.06$ ) (Figure 5F).

## 4 | Discussion

In this well-characterized cohort of 25 patients with GAD65-AIE, we evaluated the novel MAO-B PET radiotracer  $[^{18}\text{F}]\text{F-DED}$  to assess reactive astrogliosis and characterize regional uptake patterns across distinct clinical phenotypes, with the aim of establishing a surrogate marker for disease activity. Our key findings are as follows:

- GAD65-AIE patients showed globally increased  $[^{18}\text{F}]\text{F-DED}$  uptake compared to controls. Regional uptake was elevated in the MTL in LE/TLE patients and in the cerebellum of CA patients.
- $[^{18}\text{F}]\text{F-DED}$  PET imaging more frequently detected MT abnormalities in GAD65-LE/TLE than conventional routine cMRI (90.5% [19/21] with increased MT tracer uptake in PET vs. 76.2% [16/21] detection of MT T2/FLAIR hyperintensities in MRI, 52% [11/21] detection of hippocampal volume alteration, and 62% [13/21] MTL volume alterations

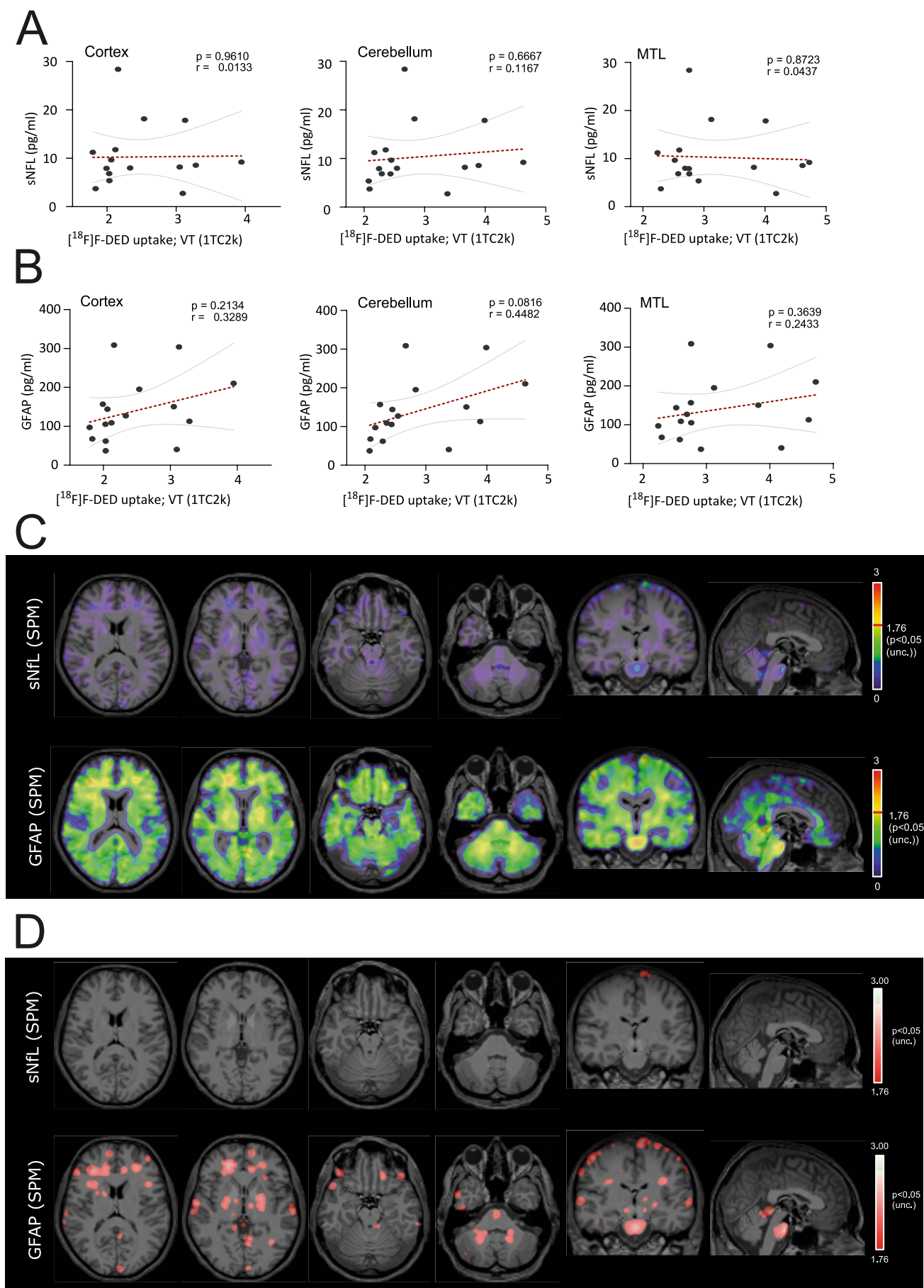


**FIGURE 4** | Legend on next page.

**FIGURE 4 |** Correlation of [ $^{18}\text{F}$ ]F-DED uptake with serum biomarkers. Correlation of [ $^{18}\text{F}$ ]F-DED uptake (volumes of distribution [VT], ITC2k model) in the cortex, mesiotemporal (MT) lobe (MTL), and cerebellar white matter with levels of serum neurofilament light chain (sNfL) (A), and serum glial fibrillary acidic protein (sGFAP) (B) was assessed using linear-regression. (C, D) Statistical parametric mapping (SPM) of VT-values was performed to identify voxel-wise positive associations with sNfL and sGFAP levels; the  $p$ -value threshold for  $t$ -scores that corresponds to  $\alpha=0.05$  is indicated by the red stroke (C). In (D) Only voxels that meet the statistically significant  $T$ -value threshold as defined in (C) are shown in color.

in MRI). Cerebellar tracer uptake in PET was observed in 87.5% [7/8] patients with GAD65-CA compared to 50% [4/8] showing cerebellar atrophy on MRI.

- No significant correlation was identified between [ $^{18}\text{F}$ ]F-DED uptake and sNfL as well as serum GAD65-Ab titers, but an association with sGFAP was noted.
- Regional [ $^{18}\text{F}$ ]F-DED signal intensity correlated with clinical severity in LE/TLE and CA phenotypes.

Emerging evidence supports a role for reactive astrogliosis in AIE. In a rat model, hippocampal astrocyte proliferation was induced following LGI1-IgG and NMDAR-IgG infusion [16]. Histopathological studies of human hippocampal tissue have similarly revealed astrocytic activation in AIE [17–19]. A recent comprehensive neuropathological study—including AIE patients with Abs against both neuronal surface and intracellular antigens (including GAD65) – demonstrated increased densities of GFAP<sup>+</sup> astrocytes and neurotoxic astrogliosis (GFAP<sup>+</sup>C3<sup>+</sup>) in the context of neuroinflammation [20]. Prior immunohistochemistry studies in mice have shown a strong correlation between GFAP<sup>+</sup> astrocytes and MAO-B expression [11]. Our findings extend these observations by demonstrating the *in vivo* detection of astrogliosis in GAD65-AIE using [ $^{18}\text{F}$ ]F-DED PET.

The observed regional correlation between [ $^{18}\text{F}$ ]F-DED uptake and clinical phenotypes, as well as areas of cMRI abnormalities [21], reinforces the specificity of the PET signal. The lack of increased cerebral tracer uptake in patients with SPS could be explained by a predominance of interneuronal spinal involvement. [ $^{18}\text{F}$ ]F-DED may serve as a valuable adjunctive tool in clinical assessment of GAD65-AIE. Our findings highlight the high prevalence of overlapping phenotypes in GAD65-AIE (9/25; 36%), supporting the concept of GAD65-associated neurological disorders as a disease spectrum [3].

Beyond aiding clinical assessment and phenotype classification, [ $^{18}\text{F}$ ]F-DED PET could be a tool for monitoring disease activity and treatment response, as it reflects disease severity in LE/TLE and CA. However, prospective studies will be necessary to adequately assess and evaluate the applicability for monitoring the response to immunotherapy. GAD65-Ab titers, although diagnostically relevant, are not considered indicative of disease activity in GAD65-AIE [3]. MRI findings are often subtle, and the disease course can be fluctuating. Thus, reliable paraclinical tools are critically needed. Effective therapeutic strategies are especially needed for patients with GAD65-LE/TLE and GAD65-CA [3]. Emerging treatment strategies include anti CD19-CART cells in SPS [22], anti-IL6 therapy (tocilizumab) in GAD65-LE [23], Janus kinase inhibitors [24], and T-cell depleting strategies [25]. A robust imaging biomarker such as [ $^{18}\text{F}$ ]F-DED PET could enable precise

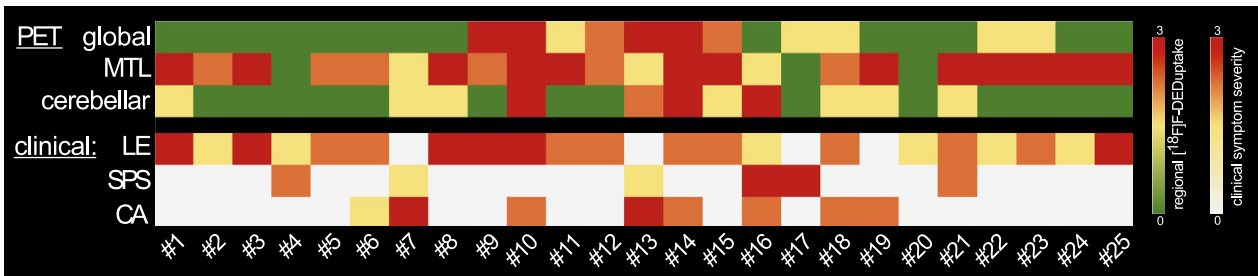
monitoring of treatment efficacy. The proposed clinical composite scores to categorize clinical severity, adapted as a shortened and GAD65-AIE tailored version of the CASE score [14], could further improve clinical monitoring. In contrast, the modified Rankin Scale (mRS), so far commonly used to assess treatment outcomes, lacks the sensitivity to detect the relevant clinical findings in this condition [26].

Although we did not observe a strong correlation between regional [ $^{18}\text{F}$ ]F-DED uptake and serum biomarkers, our prior work showed elevated sNfL levels in early-stage GAD65-AIE, particularly in CA [27]. The present study, conducted at a median disease duration of 5 years, may have missed such early sNfL elevations. Alternatively, [ $^{18}\text{F}$ ]F-DED uptake and sNfL increase may reflect different aspects of disease pathology. The lack of correlation of [ $^{18}\text{F}$ ]F-DED uptake with serum GAD65-Abs is not unexpected as GAD65-Abs are not considered to be markers of disease activity [3]. While increased sGFAP levels have been linked to disease progression in multiple sclerosis [28] and disease activity and disability in NMOSD [29], its role in AIE remains unclear. In this cross-sectional study, cerebellar [ $^{18}\text{F}$ ]F-DED uptake showed a trend toward association with sGFAP levels. Notably, voxel-wise analyses revealed an association between [ $^{18}\text{F}$ ]F-DED white matter signal and sGFAP levels. Larger studies are needed to confirm these preliminary findings including patients with early-stage GAD65-AIE and applying closer temporal alignment of biomarker evaluation and PET imaging. Together, [ $^{18}\text{F}$ ]F-DED PET along with measurement of sGFAP levels may form a useful composite for *in vivo* assessment of disease activity.

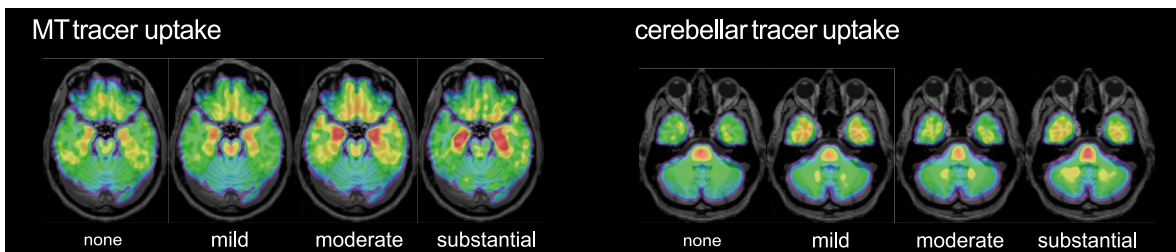
Limitations of the study include the relatively small sample size—particularly for patients with CA and SPS—and its cross-sectional design, which may introduce confounding factors such as variable disease duration, episodes of disease reactivation, and ongoing immunosuppressive therapy. The use of heterogeneous MRI protocols precluded quantitative analysis of cMRI data. The control cohort also did not match the patient cohort in terms of sex ratio. Finally, biomarker correlations should be interpreted with caution due to imperfect temporal alignment with PET imaging. Nonetheless, our pilot findings support the feasibility and clinical relevance of [ $^{18}\text{F}$ ]F-DED PET in a well-defined cohort of GAD65-AIE patients. Future longitudinal studies with larger cohorts, standardized cMRI protocols, and longitudinal analyses will help to validate these promising observations. Future studies should also assess the utility of [ $^{18}\text{F}$ ]F-DED PET in relation to other PET modalities.

In conclusion, [ $^{18}\text{F}$ ]F-DED PET represents an encouraging imaging modality for *in vivo* assessment and monitoring of astrogliosis in GAD65-AIE. It holds potential as a biomarker for disease activity and a valuable tool to guide and assess therapeutic interventions.

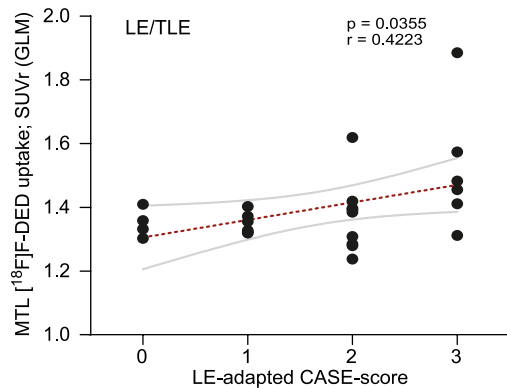
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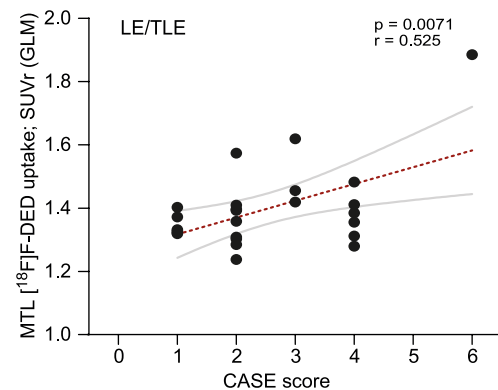
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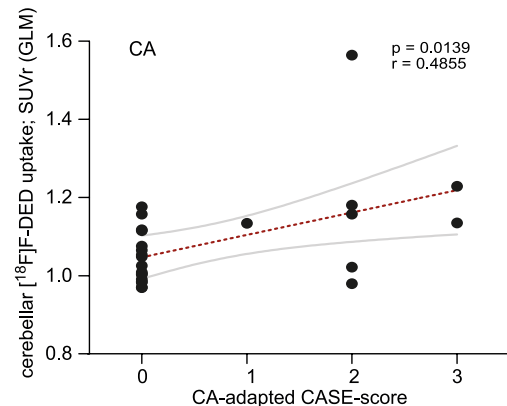
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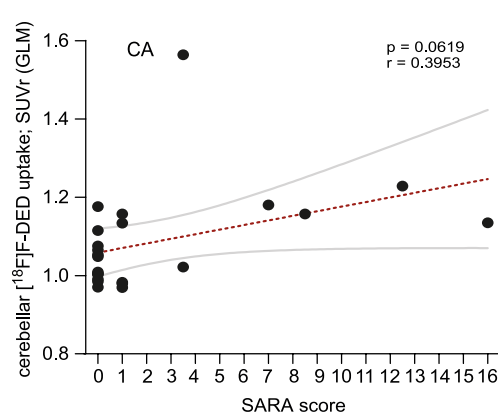


**FIGURE 5** | Correlation of regional  $[^{18}\text{F}]$ F-DED uptake with clinical phenotype and severity. (A) Heatmap showing the relationship between clinical phenotype and severity with regional  $[^{18}\text{F}]$ F-DED uptake in mesiotemporal (MT) lobe (MTL), and cerebellar white matter. Color code of  $[^{18}\text{F}]$ F-DED uptake: Green: None, yellow: Mild, orange: Moderate, red: Substantial. Color code of clinical severity: White: No symptoms, yellow: Mild symptoms, orange: Moderate symptoms, red: Substantial symptoms (reflecting grading 0–3 of LE/TLE- and CA-adapted CASE-score) (B) Representative patients showing different gradings of MT tracer uptake: None: Patient #7, mild: Patient #22, moderate: Patient #6, substantial: Patient #1. Representative patients showing different gradings of cerebellar white matter tracer uptake: None: Patient #22, mild: Patient #6, moderate: Patient #18, substantial: Patient #13. (C, D) Correlation of limbic encephalitis (LE)/temporal lobe epilepsy (TLE) clinical severity (LE-adapted case score (C), complete CASE score (D)) with MT  $[^{18}\text{F}]$ F-DED uptake (standard uptake values [SUVr] with global mean scaling) was analyzed by simple linear regression. (E, F) Correlation of CA clinical severity (CA-adapted case score (E), SARA score (F)) with cerebellar white matter  $[^{18}\text{F}]$ F-DED uptake ([SUVr] with global mean scaling) was analyzed by simple linear regression.

## Author Contributions

J.S.D. Acquisition, analysis, or interpretation of data; Statistical analysis; Drafting of the manuscript. J.A.G. Acquisition, analysis, or interpretation of data. H.Z. Acquisition, analysis, or interpretation of data. L.T. Acquisition, analysis, or interpretation of data. M.Z. Acquisition, analysis, or interpretation of data. L.S. Acquisition, analysis, or interpretation of data. L.V. Acquisition, analysis, or interpretation of data. E.K. Acquisition, analysis, or interpretation of data. J.D. Acquisition, analysis, or interpretation of data. F.L. Acquisition, analysis, or interpretation of data. G.N.B. Administrative, technical, or material support; Critical revision of the manuscript for important intellectual content. R.P. Administrative, technical, or material support; Critical revision of the manuscript for important intellectual content. B.-S.R. Administrative, technical, or material support; Critical revision of the manuscript for important intellectual content. S.L. Administrative, technical, or material support; Critical revision of the manuscript for important intellectual content. G.U.H. Critical revision of the manuscript for important intellectual content. R.A.W. Critical revision of the manuscript for important intellectual content. M.K. Study concept and design; Critical revision of the manuscript for important intellectual content. T.K. Study concept and design; Drafting of the manuscript. M.B. Study concept and design; Acquisition, analysis, or interpretation of data; Statistical analysis; Drafting of the manuscript; Study supervision. F.S.T. Study concept and design; Acquisition, analysis, or interpretation of data; Statistical analysis; Drafting of the manuscript; Study supervision. M.B. and F.S.T. contributed equally to the study.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

Data are available to qualified researchers upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Methodological comparison of [<sup>18</sup>F]F-DED uptake quantification. **Figure S2:** Evaluation of clinical phenotypes, effect of immunotherapy, and disease duration on [<sup>18</sup>F]F-DED uptake. **Figure S3:** Correlation of [<sup>18</sup>F]F-DED uptake with serum GAD65 abs. (A) Correlation of [<sup>18</sup>F]F-DED uptake volumes of distribution [VT], 1TC2k model in the cortex, mesiotemporal (MT) and cerebellar white matter with levels of GAD65 abs was assessed using linear-regression. **Figure S4:** Comparison of regional [<sup>18</sup>F]F-DED uptake with cMRI imaging results. (A) Mesiotemporal [<sup>18</sup>F]F-DED uptake was compared in LE/TLE patients (predominant or overlapping phenotype) with and without detection of mesiotemporal T2/FLAIR hyperintensities in cMRI; differences were compared by Mann–Whitney test. **Table S1:** Supplementay medication of patients at time of [<sup>18</sup>F]fluorodeprenyl-D2 PET imaging.