BRAIN
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# 'Hippocampal innate inflammatory gliosis only' in pharmacoresistant temporal lobe epilepsy

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Drug-resistant mesial-temporal lobe epilepsy is a devastating disease with seizure onset in the hippocampal formation. A fraction of hippocampi samples from epilepsy-surgical procedures reveals a peculiar histological pattern referred to as 'gliosis only' with unresolved pathogenesis and enigmatic sequelae. Here, we hypothesize that 'gliosis only' represents a particular syndrome defined by distinct clinical and molecular characteristics.

We curated an in-depth multiparameter integration of systematic clinical, neuropsychological as well as neuropathological analysis from a consecutive cohort of 627 patients, who underwent hippocampectomy for drug-resistant temporal lobe epilepsy. All patients underwent either classic anterior temporal lobectomy or selective amygdalohippocampectomy. On the basis of their neuropathological exam, patients with hippocampus sclerosis and 'gliosis only' were characterized and compared within the whole cohort and within a subset of matched pairs. Integrated transcriptional analysis was performed to address molecular differences between both groups.

'Gliosis only' revealed demographics, clinical and neuropsychological outcome fundamentally different from hippocampus sclerosis. 'Gliosis only' patients had a significantly later seizure onset (16.3 versus 12.2 years, $P = 0.005$) and worse neuropsychological outcome after surgery compared to patients with hippocampus sclerosis. Epilepsy was less amendable by surgery in 'gliosis only' patients, resulting in a significantly worse rate of seizure freedom after surgery in this subgroup (43% versus 68%, $P = 0.0001$, odds ratio = 2.8, confidence interval 1.7–4.7). This finding remained significant after multivariate and matched-pairs analysis. The 'gliosis only' group demonstrated pronounced astrogliosis and lack of significant neuronal degeneration in contrast to characteristic segmental neuron loss and fibrillary astrogliosis in hippocampus sclerosis. RNA-sequencing of gliosis only patients deciphered a distinct transcriptional programme that resembles an innate inflammatory response of reactive astrocytes.

Our data indicate a new temporal lobe epilepsy syndrome for which we suggest the term 'Innate inflammatory gliosis only'. 'Innate inflammatory gliosis only' is characterized by a diffuse gliosis pattern lacking restricted hippocampal focality and is poorly controllable by surgery. Thus, 'innate inflammatory gliosis only' patients need to be clearly identified by presurgical examination paradigms of pharmacoresistant temporal lobe epilepsy patients; surgical treatment of this subgroup should be considered with great precaution. 'Innate inflammatory gliosis only' requires innovative pharmacotreatment strategies.

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## Introduction

Drug-resistant, mesial-temporal lobe epilepsy (MTLE) is one of the most common epilepsy forms eligible for operative treatment.[1] Surgery for MTLE is considered a safe and standardized therapy option proven to have better results than conservative management in properly selected cases.[2] The most common histopathological finding after surgery for MTLE is hippocampal sclerosis (HS),[1] which is characterized by neuronal cell loss and various degrees of gliosis, is classified in three distinct groups according to the recent International League Against Epilepsy classification scheme (ILAE 1–3),[3] mainly on the basis of differences in the segmental neuronal cell loss pattern. Despite the clinical and electrophysiological evidence of a mesiotemporal seizure origin, 20% of all resected hippocampi do not reveal significant neuronal cell loss but rather a variable expression of astrogliosis and are referred to as ILAE 'no-HS'. While the first three types of HS have been investigated intensively with respect to etiopathogenetic aspects and postsurgical outcome,[1,4–8] 'no-HS' remains enigmatic in these regards.

In an MRI-negative series of patients undergoing surgery for MTLE, 'no-HS' can be even found in up to 80% of patients.[9] Recently, we reported an algorithm that enables the radiological identification of hippocampi without significant neuronal rarefication on the preoperative MRI, comprising subtle bilateral changes and less signal intensity. Thus, we concluded this pattern to represent a distinct disease entity involved in epileptogenesis.[10] Since the neuropathological hallmark of 'no-HS' is given by the lack of segmental neurodegeneration and extensive cellular astrogliosis,[11] instead of fibrillary[12] scar-type astrogliosis typically found in HS, we preferred to coin this hippocampal lesion pattern according to most prominent pathological feature 'gliosis only' and will use this term in the present study. 'Gliosis only' has, however, remained controversial since it has been also claimed to represent a 'pre-HS' stage in individual MTLE patients instead of a distinct pathological pattern.

On the basis of these precedents, we have here systematically scrutinized the hypothesis that 'gliosis only' does not only constitute a neuropathological pattern different from HS but defines a distinct MTLE form with respect to clinical and neuropathological aspects and pathomechanisms mediating epileptogenicity of the affected hippocampal formations. To approach this hypothesis, we examined demographic, neuropsychological and surgical outcome differences between two large 'gliosis only' and HS collectives. On the basis of a translational framework, we performed an integrated transcriptomic profiling, which revealed that 'gliosis only' possesses a characteristic, inflammatory-associated transcriptional signature. Taken together, our data show that 'gliosis only' resembles a distinct phenotype of MTLE, which we refer to as 'innate inflammatory gliosis only' ($I^2$GO). $I^2$GO is less curable by surgery, a finding that urgently argues for an important reconsideration of future diagnostic and clinical practice during the treatment of MTLE.

## Materials and methods

### Study population

The authors retrospectively searched the database of the Institute of Neuropathology at the University Hospital of Bonn for the results 'hippocampus sclerosis' and 'gliosis only'. A total count of 815 matched this search. Only patients with the distinct histopathological finding of 'hippocampus sclerosis' or 'gliosis only' in the hippocampal specimen, who underwent either selective amygdalohippocampectomy (sAHE) or anterior temporal lobectomy between 1990 and 2012 at the Clinic for Neurosurgery of the University Hospital at Bonn Medical Center, were included in the study (local ethical board approval 229/00). Finally, 627 patients fulfilled the eligibility criteria. Clinical data were retrospectively obtained either from patients' records or from the neurosurgical electronic database. Patients with dual pathology or other type of surgeries (e.g. disconnective procedures) were excluded ($n = 188$). All patients were evaluated and selected for surgical treatment following a standardized protocol at a tertiary epilepsy centre at the Clinic of Epileptology, University Hospital of Bonn. Only patients with drug-resistant epilepsy were included. Limbic encephalitis was excluded in all patients either by clinical or laboratory examinations. Presurgical evaluation was performed as described by Kral *et al.*[13]

### Matched-pair analysis

To avoid potential statistical confounders caused by the different sample size ('hippocampus sclerosis' = 557; 'gliosis only' = 70) a subgroup matched-pair analysis was additionally performed. For this purpose, each patient with 'gliosis only' was matched to a patient with HS by sex, type of surgery [anterior temporal lobe resection (ATL) or sAHE], side of surgery and age at surgery (with a tolerated variance of ±2 years).

## Statistical analysis

We used open-source software for statistical analysis from the jamovi project (2021: jamovi v.2.0, computer software retrieved from https://www.jamovi.org).[14] Standard procedures (Pearson, Wilcoxon and Fisher's exact tests, linear-by-linear association and Student's *t*-test) were used for univariate analyses as indicated. *P*-values <0.05 were considered to be significant. Confidence intervals (CI) are given as 95%. For multivariate analyses, we used Cox regression modelling (inclusion procedure).

## Histopathological evaluation

All histological assessments were re-evaluated for this study. The neuropathological standard procedure for epilepsy surgery specimens has been described in detail elsewhere.[15] In brief, surgical specimens were fixed in formaldehyde overnight and embedded into paraffin. Macroscopic and histopathological examinations were performed by experienced neuropathologists. The microscopic examination included haematoxylin and eosin (HE) staining and immunohistochemistry (IHC) with antibodies against neuronal nuclear specific protein (NeuN, Chemicon) and glial fibrillary acid protein (GFAP, Dako). Semiquantitative estimates of the range of hippocampal cell loss and astrogliosis were determined as described in detail in the Supplementary material.

## RNA isolation from formalin-fixed, paraffin-embedded samples

Specimens were neuropathologically re-evaluated and care was taken to have neuroanatomically optimally preserved starting material for the asservation of up to 10 serial 10 µm sections to reach equivalent amounts of starting material for all cases. HS cases that were included here, fulfilled ILEA type 1 criteria. The tubes containing formalin-fixed, paraffin-embedded (FFPE) sections for RNA purification were stored at −80°C until use. The formalin fixed samples were thawed and RNA was extracted using the RNeasy FFPE Kit (Qiagen, Cat. No. 73540) according to the manufacturer's protocol. In brief, FFPE tissue sections were first deparaffinized at 56°C for 3 min followed by lysis with proteinase K for 15 min. The genomic DNA and small fragments of DNA were removed by adding DNAse to the supernatant. Following two rounds of purification, concentrated RNA was purified using RNeasy MinElute spin columns and eluted in a volume of 30 µl of RNAase free water and stored at −80°C until use.

## Library preparation and sequencing

Since these lesions appear rarely, we used an optimized protocol to purify and sequence samples originating in some cases from up to 20-year-old paraffin embedded specimens. After thawing, the RNA concentration was measured on a Qubit 2.0 fluorometer (Thermo Fisher Scientific), and RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies). ~100 ng total FFPE RNA was selected as an input as recommended. Poly-A enriched strand specific libraries were generated using RNA TruSeq Exome Kit consisting of TruSeq RNA Library Prep for Enrichment (Illumina, Cat. No. 20020189), TruSeq RNA Enrichment (Illumina, Cat. No. 20020490) and Illumina Exome Panel Enrichment Oligos (Illumina, Cat. No. 20020183) according to manufacturer's protocol. Illumina TruSeq RNA UD Indexes (Illumina, Cat. No. 20020591) were added to each sample and ligated cDNA was selectively enriched with 15 PCR cycles. Produced libraries were then quantified using an Agilent 2100 Bioanalyzer DNA 1000 Kit (Agilent, Cat. No. 5067-1504) and a Qubit dsDNA HS Assay kit (ThermoFisher Scientific). In order to achieve a 4-plex library pool complexity, four unique precaptured cDNA libraries were combined into one pool (with a concentration of 200 ng each). Next, cDNA libraries were mixed with capture probes and hybridized probes were obtained using streptavidin magnetic beads. Captured libraries were cleaned up using AMPure XP beads (Beckman Coulter, Cat. No. A63881) before a 10-cycle PCR amplification. To block excess free adapters, Free Adapter Blocking Reagents (Illumina, Cat. No. 20024144) were added to each library pool according to the manufacturer's instructions, followed by another clean-up with AMPure XP beads. Libraries were normalized to 4 nM and combined, denatured and diluted to a final concentration of 1.1 pM. The library pool was loaded onto a MidOutput 150 cycles flowcell (Illumina, Cat. No. 20024907) and sequenced on Illumina's NextSeq 500 system. During sequencing, 65 cycles for reads 1 and 2 and 10 cycles for index 1 and 2 were used, and we obtained a cluster density of 194 K/mm$^2$. Mapped reads were normalized by DESeq.

## Transcriptional data analysis

We performed supervised identification of marker genes across both groups using the AutoPipe package (R software, CRAN) as recently described.[16] To infer functional states, we performed gene set enrichment analysis and hypergeometric testing. Astrocytic states were projected in a 2D representation using the 4state plot function of SPATA2 (https://github.com/theMILOlab/SPATA2) as recently described.[16]

## Neuropsychological assessment

Patients were neuropsychologically assessed before (T1) and one year after surgery (T2). The assessment, as previously described,[17] focuses on tests of verbal and non-verbal memory proven to be sensitive to temporal lobe pathology and the effects of temporal lobe surgery.[18–22] In addition, the assessment comprises measures of attention, executive functions, visuospatial abilities, language and motor functions. Verbal learning and memory were measured via the Verbaler Lern- und Merkfähigkeitstest[23] (VLMT), a German adaptation of the Rey Auditory Verbal Learning Test. For non-verbal learning and memory, we used the revised version of the Diagnosticum für Cerebralschädigung (DCS-R). Parallel versions of the VLMT and DCS-R were available to minimize practice effects at the follow-up. Attention was assessed by the EpiTrack and a letter cancellation task (d2 Aufmerksamkeitsbelastungstest). Language assessment comprised confrontation naming and a comprehension task (Token Test). Evaluation of motor functions included finger tapping, Luria motor task and Purdue Pegboard. The assessment consisted of visuospatial abilities by mental rotation (LPS subtest 7) and WAIS block design. The tests and their references are described in previous articles.[17,23–25]

Test results were first standardized based on age-corrected norms [mean = 100, standard deviation (SD) = 19]. In order to merge the various parameters within the respective domain, the scores were transformed into a five-point scale ranging from severely impaired to above average with the following operational definition, which has been used and published before[26,27]: severely impaired = 0, at least two test scores >2 SD below the mean of the normative sample; impaired = 1, at least two test scores >1 SD below the mean; borderline = 2, one test score below the mean; unimpaired = 3, no test score >1 SD below the mean; and above average = 4, at least two test scores

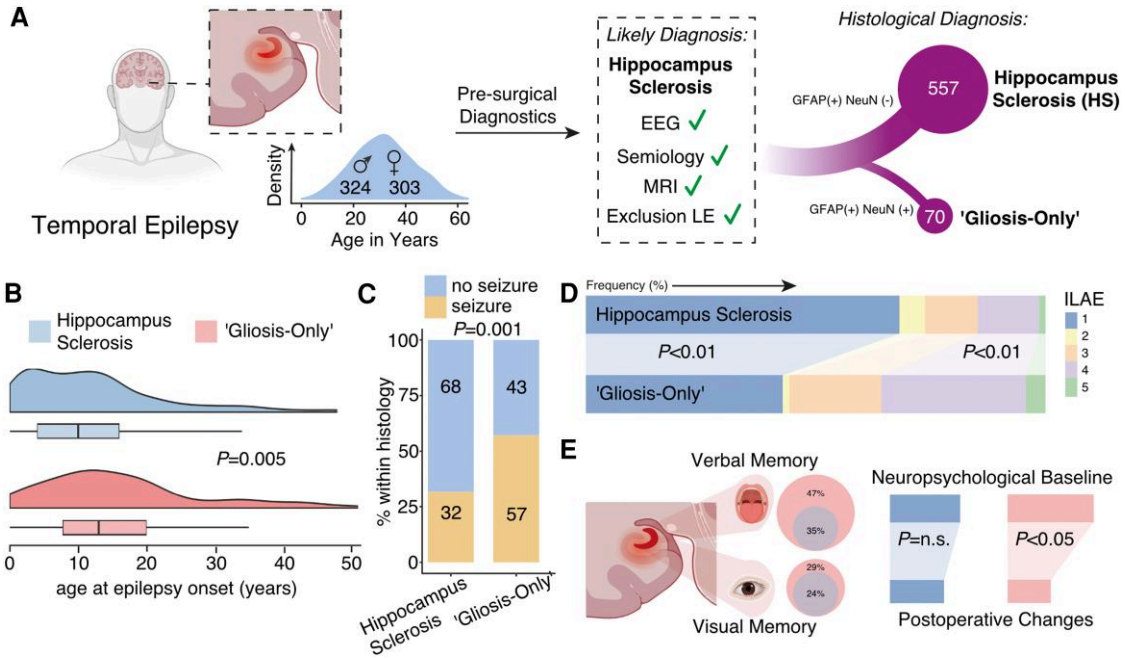


**Figure 1 Demographics and clinical results.** (**A**) Illustration of the workflow. A density plot at the *bottom right* indicates the age and gender distribution of all patients. Patient characteristics and distribution of histopathological findings after surgical resection for drug-resistant MTLE (LE = limbic encephalitis). (**B**) Patients with histopathological gliosis only developed epilepsy significantly later in life (seizure onset 12.2 years for HS versus 16.3 years for 'gliosis only', $P = 0.005$, one-sided *t*-test.). (**C**) Bar plot illustrates the postoperative epilepsy outcome in relation to neuropathological finding, Fisher's exact test, $P = 0.001$. (**D**) Seizure outcome according to the ILAE classification, showing significant better postsurgical outcome in HS than 'gliosis only', one-way ANOVA. (**E**) Graphical summary of neuropsychological results—patients with 'gliosis only' reveal significant postoperative impairment in verbal and visual memory in relation to the preoperative baseline.

>1 SD above the mean. The distance between two subsequent categories approximately corresponds to 1 SD from the mean standardized score across all test scores of the respective domain.[28] Neuropsychological change after surgery was defined as the intra-individual change in cognitive performance from pre- to postoperative assessment; the postoperative score was subtracted from the preoperative score in each domain. A positive value indicated improvement; a negative value indicated deterioration; a value of zero indicated no change.[17] Neuropsychological analysis was conducted for the matched-pairs sample. We used Chi-squared tests to assess preoperative differences and repeated-measures ANOVAs for postoperative changes on a group level with pathology and surgical side as between-subjects factors, as well as Chi-squared tests to assess individual postoperative changes.

### Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## Results

### Study cohort

A total of 627 patients ($n = 324$ female, 52%) with diagnosed drug-resistant MTLE due to presumed HS underwent standardized presurgical evaluation and were selected for surgical treatment (Fig. 1A). The complete diagnostic included at least 24 h long-term video-EEG, MRI and neuropsychological evaluation. Any clinical uncertainty for limbic encephalitis was ruled out by examination of antineuronal or onconeuronal antibodies and complete patient examination for any unknown underlying tumour. None of the patients fulfilled the relevant integrated criteria for limbic encephalitis based on clinical, MRI as well as serological and neuropathological parameters.[29,30] The median duration of epilepsy was 22 [interquartile range (IQR) 18] years and median age of seizure onset was 11 (IQR 12) years. Invasive diagnostics was performed in 281 (44%) cases. A total of 497 (79%) patients were operated by sAHE, the remaining patients underwent standard ATL. The mean postoperative follow-up was 64.7 months. HS was found in 557 patients. 'Gliosis only' was diagnosed in the remaining 70 patients.

### Differences between HS and I$^2$GO—seizure onset and seizure outcome

Due to the lack of significant cell loss, 'gliosis only' might be considered as a precursor state of early-onset HS. The demographic profiles of both cohorts described here, clearly argue against this concept. 'Gliosis only' patients developed epilepsy significantly later in life compared to their HS counterparts [median seizure onset: I$^2$GO = 13 (IQR 12.25) years versus HS = 10 (IQR 12.0) years; $P = 0.005$ two-sided *t*-test, Fig. 1B and Table 1], supporting the hypothesis that 'gliosis only' constitutes a distinct clinical–pathological condition.

Of note, anticonvulsive drug-resistance was reached in 'gliosis only' patients far more rapidly, consequently leading to an earlier inclusion for presurgical evaluation that resulted in significantly shorter duration of epilepsy [median duration of epilepsy: I$^2$GO = 20 (IQR 17.5) years versus HS = 22 (IQR 19) years; $P = 0.005$].

**Table 1 Cohort description and univariate analysis**

| Parameter/condition | Sclerosis | I²GO[b] | Test statistic |
|---|---|---|---|
| **Whole cohort** | **(*n* = 557)** | **(*n* = 70)** | |
| Sex: female | 0.5 288/557 | 0.5 36/70 | *P* = 0.97[a] |
| Onset of epilepsy, years | 4.0 **10.0** 16.0 | 7.4 **13.0** 20.0 | **P < 0.01**[b] |
| Duration of epilepsy, years | 14.0 **22.0** 33.0 | 8.4 **20.0** 26.6 | **P < 0.01**[b] |
| Age at surgery, years | 28.0 **37.0** 45.3 | 24.9 **34.0** 44.1 | *P* = 0.08[b] |
| Side of surgery: right | 0.5 261/557 | 0.5 38/70 | *P* = 0.24[a] |
| Type of surgery: sAHE | 0.8 466/557 | 0.4 31/70 | **P < 0.01**[a] |
| Invasive EEG: no | 0.6 324/555 | 0.3 20/70 | **P < 0.01**[a] |
| Outcome: seizure ILAE2-6 | 0.3 177/557 | 0.6 40/70 | **P < 0.01**[a] |
| **Matched pairs** | **(*n* = 70)** | **(*n* = 70)** | |
| Sex: female | 0.5 36/70 | 0.5 36/70 | *P* = 1.00[a] |
| Onset of epilepsy, years | 3.0 **9.0** 14.0 | 7.4 **13.0** 20.0 | **P < 0.01**[b] |
| Duration of epilepsy, years | 16.0 **23.5** 31.1 | 8.4 **20.0** 26.6 | **P = 0.01**[b] |
| FBTCS: yes | 0.4 29/70 | 0.6 41/70 | **P = 0.04**[a] |
| Seizures | | | |
| Focal aware | 0.2 14/70 | 0.3 19/70 | |
| Focal unaware | 0.5 32/70 | 0.5 36/70 | |
| Aware + unaware focal | 0.3 24/70 | 0.2 15/70 | *P* = 0.22[a] |
| Interictal EEG | | | |
| bilateral features: yes | 0.3 20/70 | 0.3 19/70 | *P* = 0.85[a] |
| Ictal EEG | | | |
| bilateral features: yes | 0.4 27/70 | 0.3 18/70 | *P* = 0.14[a] |
| Invasive EEG: yes | 0.6 39/70 | 0.7 50/70 | **P = 0.05**[a] |
| Invasive interictal EEG | | | |
| bilateral features: yes | 0.1 4/39 | 0.0 2/50 | *P* = 0.39[c] |
| Invasive ictal EEG | | | |
| bilateral features: yes | 0.3 10/39 | 0.1 5/50 | *P* = 0.09[c] |
| Early childhood convulsion: yes | 0.3 22/70 | 0.4 28/70 | *P* = 0.29[a] |
| Traumatic brain injury: yes | 0.0 1/70 | 0.1 5/70 | *P* = 0.1[a] |
| Age at surgery, years | 25.0 **35.5** 43.1 | 24.9 **34.0** 44.1 | *P* = 0.82[b] |
| Side of surgery: right | 0.6 39/70 | 0.5 38/70 | *P* = 0.87[a] |
| Type of surgery: sAHE | 0.5 32/70 | 0.4 31/70 | *P* = 0.87[a] |
| Outcome: seizure: ILAE 2–6 | 0.4 25/70 | 0.6 40/70 | **P = 0.01**[a] |

Cross table for univariate testing with categorial and continuous parameters for histology as the dependent variable. In the whole cohort parameters are calculated for the whole cohort (*n* = 627), while in matched-pairs parameters are calculated for the matched pair (*n* = 140) analysis. For continuous parameters the median value is printed bold and framed by its first and third quartiles (e.g. 4.0 10.0 16.0). Outcome is calculated for persisting epilepsy ILAE class 2–6. FBTCS = focal to bilateral tonic clonic seizure.
[a]Pearson.
[b]Wilcoxon.
[c]Fisher's exact test.

Univariate testing for categorical and continuous parameters regarding demographics is shown in Table 1.

Because of the skewed distribution between HS and 'gliosis only' patients, we additionally performed a matched-pairs analysis including detailed patients' characteristics. We matched patients with 'gliosis only' by gender, type and side of surgery and age at surgery (with a tolerance of ±2 years) to a sclerosis counterpart and formed 70 pairs. In line with the results of the whole cohort analysis, seizure onset and duration of epilepsy showed significant differences between both groups, while other clinical characteristics (e.g. prior neurological insults, early childhood seizures, family history, interictal and ictal EEG) did not separate HS and I²GO.

One of the main clinical findings distinguishing I²GO from HS patients was the final seizure outcome. In general, surgery for MTLE is a successful treatment option with excellent seizure outcome. However, I²GO patients represent an important exception. At the last available outcome, only 43% (*n* = 30) of the I²GO patients were seizure free (ILAE1) compared to 68% (*n* = 380) of the HS cases [*P* = 0.0001, odds ratio (OR) = 2.8, CI 1.7–4.7)] (Fig. 1C). This finding remained significant for other ILAE classes as well (Fig. 1D).

## I²GO is associated with unfavourable seizure outcome

Since other factors can influence final seizure outcome as well, we performed a multivariate logistic regression analysis testing the hypothesis that the underlying histology independently influences patients' outcomes. The analyses for the whole cohort (*n* = 627) confirmed 'gliosis only' (*P* < 0.0001, OR = 2.98, CI 1.73–5.16) as an independent predictor for worse seizure outcome after correcting for other variables known to influence the seizure outcome (Table 2, left panel, whole cohort analysis).

In line with these findings, histology of 'gliosis only' was also the only independent predictor, associated with worse seizure outcome in the multivariate matched-pair analysis (*P* = 0.030, OR = 2.182, CI 1.075–4.427, Table 2, right panel). Two additional multivariate models including further clinical variables (early childhood seizures, meningitis, number of anti-seizure medications and presence of focal to bilateral tonic clonic seizures) confirmed this finding showing a significant association between 'gliosis only' and poor seizure outcome (Supplementary Table 1).

**Table 2 Binominal logistic regression for ILAE1 outcome**

| Predictor | Whole cohort (total $n = 627$; HS $n = 557$) | | | | Matched pair (total $n = 140$, HS $n = 70$) | | | |
|---|---|---|---|---|---|---|---|---|
| | P | Odds ratio | 95% CI | | P | Odds ratio | 95% CI | |
| | | | Lower | Upper | | | Lower | Upper |
| Side of surgery Right—Left | 0.3132 | 0.820 | 0.557 | 1.205 | 0.916 | 1.038 | 0.512 | 2.116 |
| Duration of epilepsy | 0.3521 | 1.007 | 0.992 | 1.022 | 0.389 | 0.987 | 0.958 | 1.017 |
| Type of surgery sAHE—ATL | 0.2380 | 1.338 | 0.824 | 2.173 | 0.530 | 0.798 | 0.396 | 1.611 |
| Invasive EEG No—Yes | **0.0378** | 0.660 | 0.446 | 0.976 | 0.640 | 1.188 | 0.575 | 2.453 |
| Histology 'gliosis only'—HS | **0.0009** | 2.706 | 1.505 | 4.865 | **0.030** | 2.182 | 1.075 | 4.427 |

A binominal logistic regression analysis for the whole cohort ($n = 627$) and the matched-pair cohort ($n = 70$ pairs = 140 patients) with ILAE1 seizure outcome as the dependent variable. For the whole cohort, invasive EEG and the histology finding of gliosis only were independent prognostic factors for a worse seizure outcome. Within the matched-pair group, the analysis confirms 'gliosis only' as the only independent prognostic factor for worse seizure outcome while invasive EEG lost its influence for the seizure outcome. Estimates represent the log odds of 'outcome = seizures' versus 'outcome = seizure free'. Significant values are highlighted in bold.

## Gliosis only in I²GO differs from HS gliosis pattern

Neuropathologically, 'gliosis only' differs fundamentally from HS by neuronal cell density, fibrillary as well as cellular astrogliosis (Fig. 2).

In 'gliosis only', i.e. the neuropathological surrogate of I²GO, the combination of largely conserved neuronal densities accompanied by mainly a cellular reactive astrogliosis throughout all subfields is characteristic. The lesion pattern is in striking contrast to HS with pronounced segmental neurodegeneration in CA1, CA3 and CA4, whereas neuronal densities in CA2 and the dentate gyrus granular layer are rather conserved. Granule cell dispersion is seen in the HS pathology pattern (Fig. 2). Accordingly, semiquantitative neuron to glia ratios provide distinct fingerprints separating all different anatomical regions between the 'gliosis only' and HS patterns (Supplementary Fig. 2).

## I²GO is associated with a greater risk for cognitive decline

Complete neuropsychological data sets from before and after surgery were available for 46 patients with HS and 62 patients with 'gliosis only'. Before surgery, at baseline, 90% of the patients with HS and 'gliosis only' were impaired in at least one cognitive domain. Memory and language were most frequently affected (Table 3). Of note, 'gliosis only' patients showed slightly fewer impairments across the domains, and memory impairments tended to be less lateralized than in HS patients.

On a group level, verbal [$F(1,107) = 6.96$, $P < 0.05$] and non-verbal memory [$F(1,107) = 4.01$, $P < 0.05$] differed significantly between right and left TLE. There were no significant differences in attention, motor function and visuospatial abilities.

After surgery, 'gliosis only' patients declined more frequently in verbal memory (64%), and language (25%) after left TLR, and in non-verbal memory (26%) after right TLR than HS patients, who declined in 30, 15 and 17%, respectively. Extratemporal functions (attention, motor functions) also deteriorated more frequently in 'gliosis only'. Verbal memory decline was twice as likely in gliosis only than in HS [$\chi^2(2) = 7.14$, $P = 0.03$]. A decline in language was more likely in 'gliosis only' than in HS [$\chi^2(2) = 5.07$, $P = 0.08$].

Repeated-measures ANOVAs revealed a significant main effect of surgery on the group level. Following resection, both groups showed cognitive decline in verbal [$F(1,104) = 7.19$, $P < 0.05$] and non-verbal memory [$F(1,104) = 6.71$, $P < 0.05$]. Attention [$F(1,103) = 7.49$, $P < 0.05$] and visuospatial abilities [$F(1,94) = 7.83$, $P < 0.05$] improved rather than declined. Language and motor functions did not show significant postoperative changes on a group level.

## I²GO shows a unique gene-expression signature

Using RNA-sequencing we profiled 32 histologically defined HS and 'gliosis only' specimens. To avoid age and gender bias, we matched the samples on the basis of their clinical features. Out of 32 specimens, 24 reached quality control after library construction (Fig. 3A). The high dropout is caused by the fact that the tissue was up to 20 years old (paraffin embedded), which posed a challenge for RNA-sequencing. After combining unsupervised clustering with supervised analysis of differential expressed genes and correction for multiple testing (FDR) we identified a stable set of 265 genes, which marked the differences between classical HS and 'gliosis only' (Fig. 3B). We observed several transcripts encoding proteins with inflammatory-relevant function that were significantly upregulated in the 'gliosis only' including Apolipoprotein E (*APOE2*), C-C Motif Chemokine Ligand 2 (*CCL2*), Interleukin 1 Alpha (*IL1A*), Macrophage-Associated Antigen (*CD163*) and complement factors. Using gene set enrichment analysis, we confirmed an increase of inflammatory response and activation of the complement system (Fig. 3C). Additionally, other markers such as *CD3D*, Hepatitis A Virus Cellular Receptor 2 (*HAVCR2*) and Programmed Cell Death 1 (*PDCD1*) that are known hallmarks of chronic inflammation were significantly expressed in 'gliosis only' (Fig. 3C). To further classify our samples, we computed a two-dimensional classification model, which showed a shift of 'gliosis only' samples towards the signature genes defining the inflammatory state (Fig. 3D). This was further confirmed by aligning the inflammatory score to clinical features which revealed an exclusive inflammatory enhancement in the 'gliosis only' group not biased by other clinical parameters (Fig. 3E).

Since the major subtype of cells was reactively transformed astrocytes, we aimed to explore the linkage between 'gliosis only' astrocytes and common reactive subtypes. Using an unsupervised clustering of publicly available datasets of astrocytes from different CNS diseases and our transcriptional data, we were able to align our samples to known reactive subtypes. The transcriptional profile of HS clustered within a non-inflammatory reactive state similar to reactive astrocytes found in stroke or glial tumour samples. In contrast,

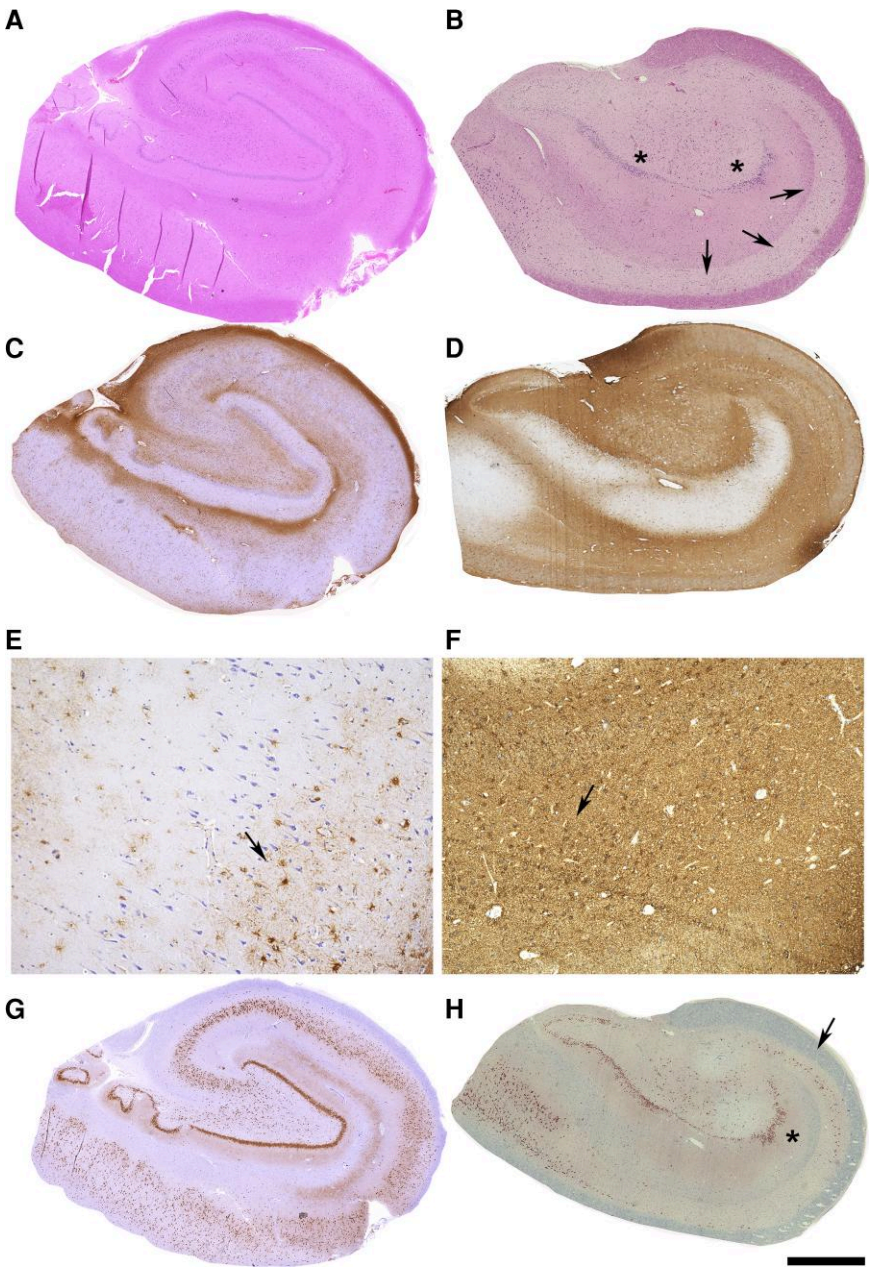


**Figure 2 Neuropathological differences in 'gliosis only' versus HS.** (**A**) Hippocampal formation with the lesion pattern referred to as I²GO; note the virtual absence of neurodegeneration (HE staining). (**B**) HS ILAE type 1 with extensive segmental neurodegeneration pronounced CA 1 (arrows), CA3 and CA4 (asterisks). (**C**) Mainly cellular astrogliosis of the hippocampus is detected in I²GO (IHC with antibodies against GFAP). (**D**) Intense fibrillary astrogliosis predominates in HS (GFAP-IHC). (**E**) Reactive astroglial cells with large somata and delicate stellate processes are present in varying density virtually throughout all layers of the representative CA1 area high power magnification (GFAP-IHC); astroglial cells are occasionally clustered (arrow). Note that the neuronal density is largely conserved. (**F**) Higher power magnification in HS-CA1 reveals the presence of an extensive fibrillary astroglial matrix, which constitutes a scar-resembling pattern admixed to only rather sparse reactive astrocytes (arrow; GFAP-IHC). (**G**) NeuN-IHC underlines the virtual absence of neuronal loss in I²GO. (**H**) NeuN-IHC emphasizes granule cell dispersion (asterisk) and conservation of CA2 neurons (arrow) in addition to subtotal neurodegeneration in CA1 and CA3/4 in HS (bar graph corresponds to 1000 µm in **A–D**, **G** and **H**; 200 µm in **E** and **F**).

the histopathological 'gliosis only' showed astrocytes state similar to those observed in partially inflammatory diseases (Supplementary Fig. 1).

## Discussion

Surgery for drug-resistant MTLE is a safe, standardized and effective treatment option. However, it fails to achieve seizure freedom in 30–40% of the patients, suggesting that the underlying lesion of the mesial-temporal structures may not entirely cover the epileptogenic zone. 'Gliosis only', as we have recently coined this neuropathological pattern,[10] is a finding occurring in ~20% of the patients with MTLE included in the current ILAE classification as 'no-HS'. Our results support the hypothesis that 'gliosis only' hallmarks a distinct disease entity with inflammation as underlying background, which we refer to as I²GO. I²GO is defined by a specific neuropathological

**Table 3 Neuropsychological performance before and after surgery**

| | | Left | | | | Right | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Impaired (T1) | Impaired (T2) | Losses | Gains | Impaired (T1) | Impaired (T2) | Losses | Gains |
| Attention | I²GO | 61% | 50% | 11%[a] | 25% | 50% | 50% | 16%[a] | 23% |
| | HS | 70% | 32% | 5%[a] | 45% | 48% | 52% | 9%[a] | 26% |
| Verbal memory | I²GO | 75%[a] | 89% | **64%** | 14% | 65%[a] | 65% | 32%[a] | 39% |
| | HS | 87%[a] | 96% | **30%** | 13% | 61%[a] | 78% | 23%[a] | 22% |
| Non-verbal memory | I²GO | 54% | 68% | 36% | 11% | 77% | 84% | 26%[a] | 10% |
| | HS | 70% | 74% | 30% | 13% | 74% | 87% | 17%[a] | 13% |
| Language | I²GO | 71% | 79% | 25%[a] | 4% | 67% | 53% | 14%[a] | 31% |
| | HS | 77% | 80% | 15%[a] | 25% | 73% | 67% | 29%[a] | 33% |
| Visuospatial abilities | I²GO | 52% | 48% | 19% | 15% | 34% | 24% | 7% | 25% |
| | HS | 45% | 30% | 20% | 45% | 57% | 48% | 10% | 45% |
| Motor functions | I²GO | 60% | 57% | 35%[a] | 30% | 57% | 50% | 22% | 33% |
| | HS | 70% | 63% | 11%[a] | 32% | 63% | 64% | 15% | 23% |

T1 = preoperative; T2 = postoperative; losses/gains = change of at least 1 SD from pre- to postoperative performance. Significant differences are highlighted in bold.
[a]Indicates a trend, but did not reach significance with $P < 0.05$.

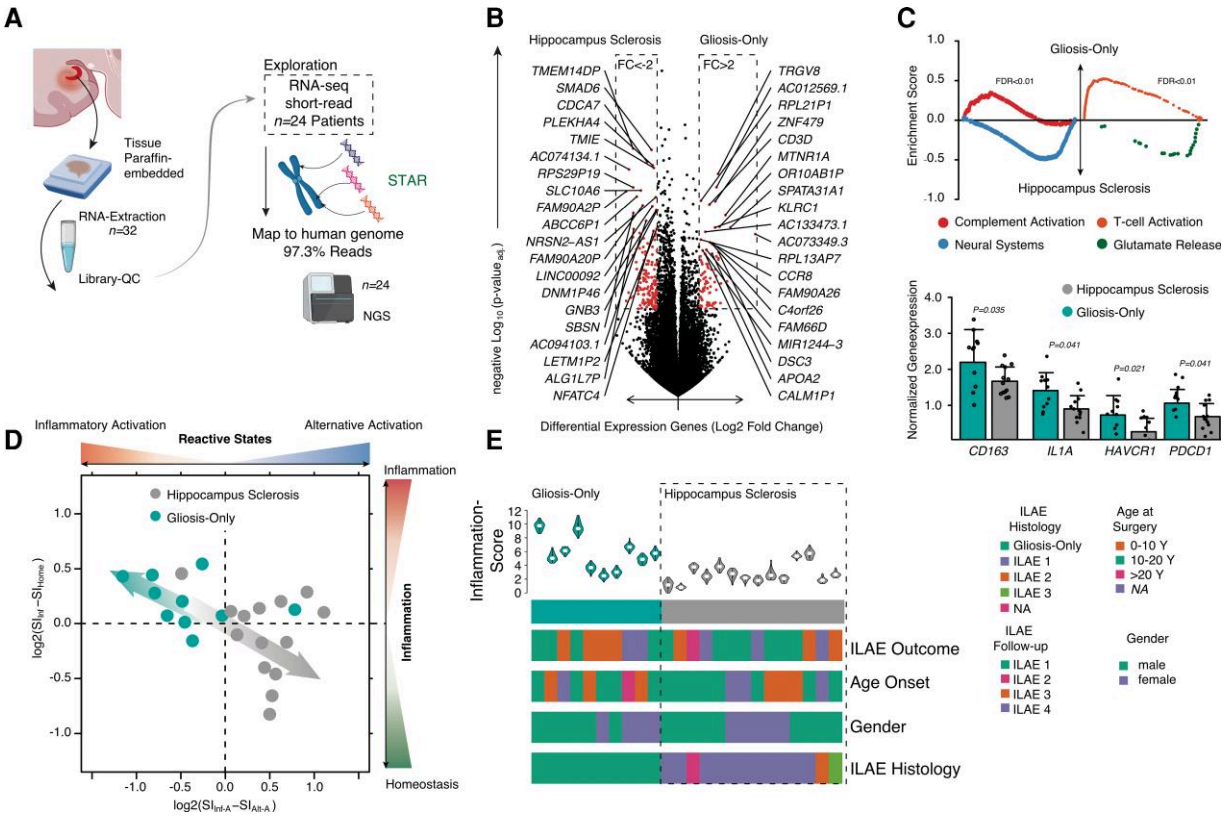


**Figure 3 Transcriptional signature and gene expression.** (**A**) Illustration of the workflow. (**B**) Differential gene-expression analysis presented as a volcano plot. (**C**) Gene set enrichment analysis from the MSigDB (v.7.0) indicate significant enrichment of the complement and inflammatory response in 'gliosis only' samples and an upregulation of neuronal systems and glutamate release in HS samples, Kolmogorov–Smirnov-like test with adjustment of *P*-value using the false discovery rate. At the *bottom*, bar plots of gene-expression differences between 'gliosis only' and sclerosis samples using normalized gene-expression values. Wilcoxon rank with adjustment of the *P*-value by Benjamini–Hochberg. (**D**) 2D representation of astrocytic transformation. Each quadrant corresponds to a defined substate of reactive astrocytes, the illustrated position of each transcriptome reflects their relative scores for inflammatory-alternative activation (*x*-axis) and their grade of differentiation between adult and foetal programmes (*y*-axis). (**E**) Violin plot (*top*) indicates the individual inflammatory score calculated from mean expression of genes with inflammatory signatures with respect to the clinical information illustrated at the *bottom*.

pattern dominated by cellular gliosis, which renders a specific transcriptomic profile and follows a characteristic demographic and clinical patterns (Fig. 4) making it less curable by surgery supporting the inflammatory nature of the disease.

In line with these findings, two recent imaging studies have revealed characteristic structural and connectivity MRI patterns distinguishing 'gliosis only MRI-negative'- and 'HS-caused'-MTLE.[10,31] Recently, our group published an MRI study showing that 'gliosis

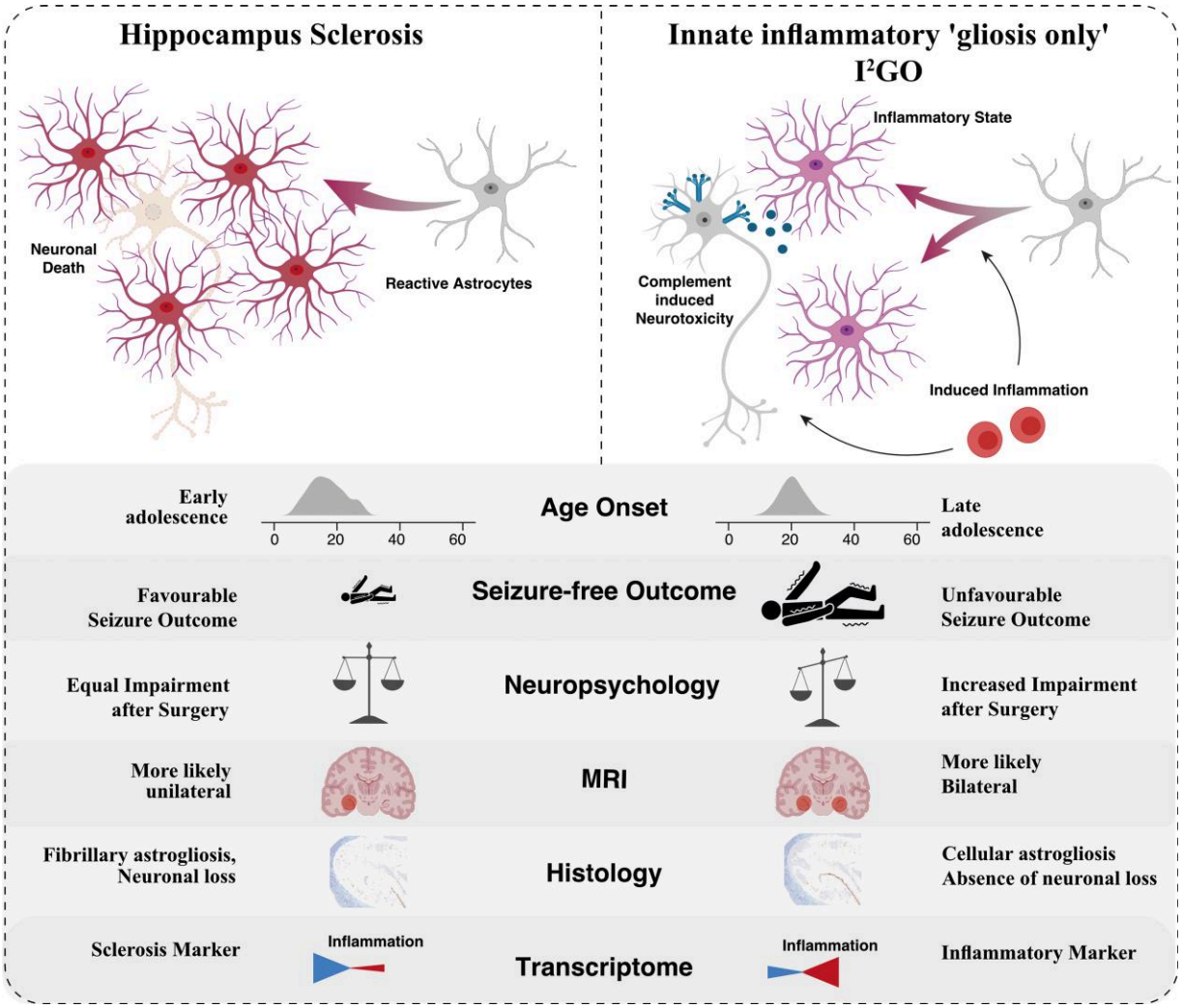


**Figure 4 Graphical summery of the differences between hippocampal I²GO and HS.** I²GO constitutes a distinct MTLE syndrome with characteristic clinical and pathological features. I²GO is less amendable by surgery and bears a greater hazard for postoperative neuropsychological deterioration. 'Gliosis only', the neuropathological hallmark of I²GO, shows a unique transcriptional signature marked by an astrocyte-mediated chronic inflammation pattern.

only' reveals characteristic MRI features discriminating it from HS.[10] Therefore, we used the same MRI criteria to evaluate a representative subset of MRI images of patients with 'gliosis only' and HS. The results supported the previous results, showing that characteristic of HS features (reduction in hippocampal volume, complete loss of internal hippocampal structure and the marked increase in $T_2$-signal intensity) were absent in most I²GO cases (Supplementary Fig. 3). These findings were further confirmed by a quantitative assessment of hippocampus and amygdala volumes and normalized fluid-attenuated inversion recovery (FLAIR) signal showing significant differences between affected hippocampus and contralateral hippocampus in HS. In contrast, no significant difference could be found between the affected and the contralateral hippocampus in patients with I²GO (Supplementary Figs 4 and 5).

## I²GO patients have distinct demography and worse seizure and neuropsychological outcome compared to HS patients

Here we argue that I²GO neuropathologically hallmarked by 'gliosis only' may represent a distinct disease entity mapping to a characteristic phenotype. Patients with I²GO are significantly older than their HS counterparts, which *per se* excludes the hypothesis that 'gliosis only' is a precursor state of HS.

Patients with I²GO tended to show less and more diffuse cognitive impairments prior to surgery compared to HS. Verbal and non-verbal memory more frequently declined in this group after surgery. Memory performance in TLE very much depends on the structural and functional integrity of the hippocampus.[32] The finding of more diffuse and less severe memory impairment in I²GO would be in line with the assumption of a less severe, more diffuse and more bilateral hippocampal pathology. This puts this group at a greater risk of postoperative decline.[32]

Together with the fact that I²GO patients are less likely to become seizure free, they are at a higher risk of becoming so-called 'double losers', i.e. not becoming seizure free and also experiencing memory loss.

## I²GO epileptogenicity based on astrocytic induced inflammation without neuronal cell loss

The findings so far suggest that in I²GO, different from HS, a less severe, more diffuse and widespread pathology is being found, implicating a more widespread epileptogenic zone with greater risk for

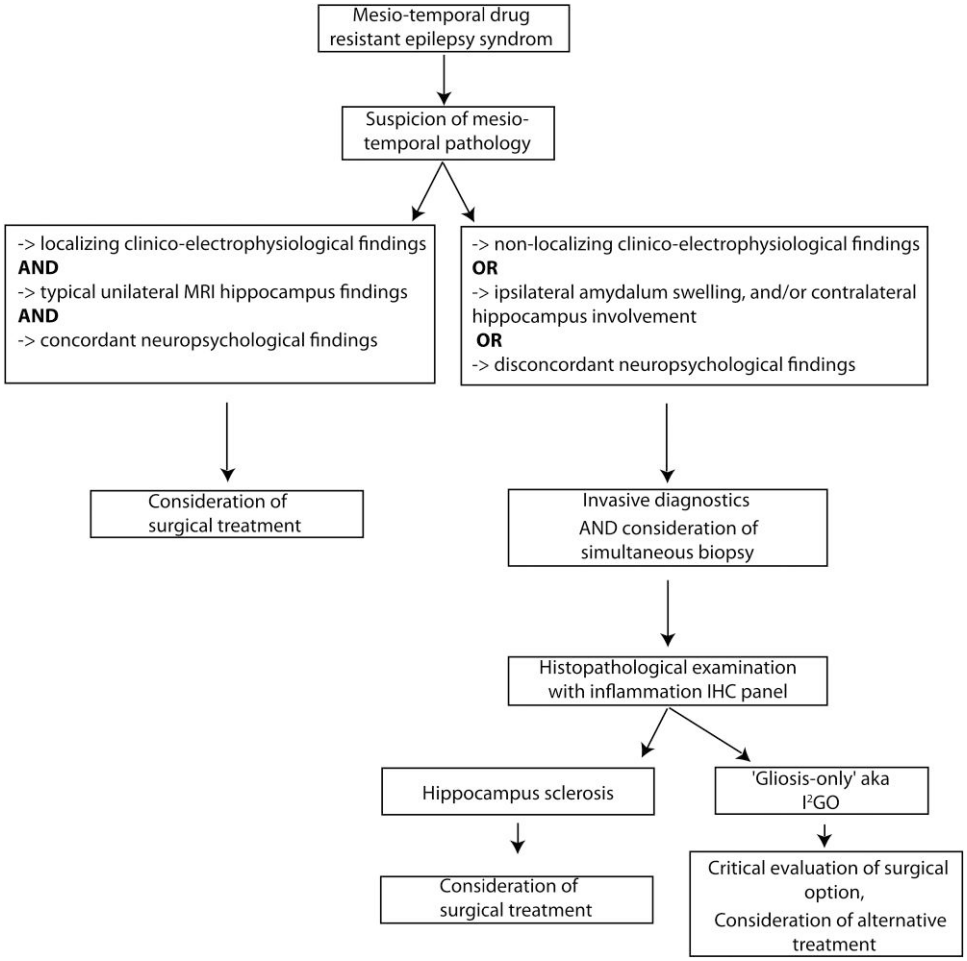


**Figure 5 Decision pathway considering the diagnosis of I²GO as part of the presurgical diagnostics.**

seizure relapse after standard surgical procedures. Presurgical analyses as well as neuropsychological focus mapping have clearly ruled out epileptogenic network activity outside the hippocampal formation as the seizure onset zone in the present patients. If a suspicion of extratemporal or even temporal lateral focus was raised during the preoperative evaluation, patients were suggested for invasive diagnostic tests aiming to localize the focus precisely. Therefore, the observed gliosis pattern constitutes an intrinsic pathological aspect of the epileptogenic focus. In this respect, it is remarkable that the comparison of transcriptional programmes between both entities reveals a first hint of potential innate non-adaptive inflammatory alterations suggesting a distinct pathomechanism in I²GO. Pathogenetically, the molecular profile of the I²GO hippocampi uncovers a strongly inflammatory micromilieu evoked by the reactively transformed astroglial cell component that is therefore well suited to fundamentally contribute to epileptogenesis of the affected hippocampal network. We observed a distinct activation of the complement pathway associated with inflammatory adaptation of astrocytes similar to those observed in inflammatory diseases such as Morbus Alzheimer or encephalomyelitis disseminate (Supplementary Fig. 1). Further investigation is required to corroborate these initial suspicions. In addition, the frequent bilateral occurrence of I²Go underpinning these systemic inflammatory changes may be involved in disease pathogenesis. Thus, we assume that the reactive astrocytes in I²Go drive aberrant neuronal plasticity, which is constituted by an astrocyte-neuron signalling cascade resulting in persistent functional modification of hippocampal excitatory synapses.[33]

In our work, we linked inflammatory transcriptional programmes to patients with a significantly worse clinical outcome in terms of seizure freedom and neuropsychology, although the causality was not proven and need further experimental validations. Other authors have demonstrated a neurotoxic effect of reactive astrocytes on the hippocampus in murine models. Although the definite mechanism remains to be further examined, it can be assumed that alterations in neuronal synapses are provoked by a loss of homeostatic functions and release of inflammatory cytokines.[33] Thus, our results reveal abundant transcriptional differences between HS and I²GO, suggesting two different disease entities. The neuroinflammatory transcriptional signature of I²GO suggests more global and vaster pathomechanisms involved in epilepsy development, which may be less amendable by surgical treatment. In accordance, the clinical differences between both groups supported the transcriptional results.

## Clinical implications

Beside all other differences, it is the significantly worse postoperative seizure outcome combined with the higher risk for neuropsychological deterioration after surgery that urges a direct clinical consequence. Figure 5 proposes a decision-making flow chart,

which considers the diagnosis of 'gliosis only' before performing a resection of mesial-temporal structures. For this purpose, any inconclusive non-invasive findings during the preoperative diagnostics of MTLE, which lead to the indication of invasive electrode implantation, should be critically evaluated under the spotlight of the current results. In particular, in patients showing ipsilateral amygdala swelling and/or contralateral hippocampus involvement as well as less severe and more diffuse preoperative neuropsychological impairment, a biopsy of a tissue sample to exclude possible 'gliosis only' together with invasive EEG, should be taken into consideration. The biopsy samples are mainly encountering hippocampal tissue (CA1, CA3 and CA4) that reflect the maximal cellular compositional—and therefore—transcriptional profiling differences between $I^2GO$ and HS. Therefore, it may be clearly anticipated that the neuropathological in concert with the mRNA signature analyses in biopsy specimens will successfully differentiate $I^2GO$ from HS. Consequently, if 'gliosis only' is diagnosed, further conservative treatment options prior to surgery should be critically discussed with the patients and their caregivers.

This algorithm should not be interpreted as scepticism towards surgical treatment of patients with MTLE, but rather as 'change of paradigm' with 'red flags' pointing at important implications for the consultation and treatment of patients with one of the most common epilepsy types. Even though most patients with TLE can be classified according to the established syndromic groups, there are still subfractions of patients with TLE, where the pathology and pathogenesis are still difficult to define, and conversely, also defining clear and unequivocal clinic-electrophysiological/MRI profiling features remains somewhat vague. Especially in ambiguous cases, the use of radiologic biomarkers including quantitative volumetric analysis, estimation of $T_2$ relaxation time through the hippocampus or assessment of normalized FLAIR signal may help to clarify the diagnosis of $I^2GO$, thus supporting the decision-making process during routine preoperative work-up.[34,35] Concerning the topic of TLE, this e.g. holds true for grey-white matter blurring.[36] However, molecular genetic studies may fundamentally improve the categorization of patients with so far poorly defined epilepsy and support the improved definition of epilepsy-associated syndromes. The finding of abundant SLC35A2 brain mosaicism in mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy may be regarded as a striking example in this context.[37] Our present paper clearly shows that the integration of neuropathological features with a transcriptomic signature fundamentally fosters the definition of an epilepsy syndrome ($I^2GO$, as we suggest here) overcoming the rather descriptive 'no-HS, 'gliosis only' in a TLE patient group that has so far been difficult to define by integrated clinico-electrophysiological/MRI and histological characteristics. $I^2GO$ is less curable by surgery. Therefore, adequate treatment requires a revision of the current MTLE diagnostic and clinical practice algorithm and the consideration of novel pharmacotherapies (e.g. fingolimod)[38] in the future.

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## Competing interests

The authors report no competing interests.

## Supplementary material

Supplementary material is available at *Brain* online.

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