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The epileptology of Aicardi-Goutières syndrome: electro-clinical-radiological findings

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ABSTRACT

Objective: Although epileptic seizures occur in approximately a quarter of patients with Aicardi-Goutières syndrome (AGS), their phenotypic and electrophysiological characterization remains elusive. The aim of our study was to characterize epilepsy phenotypes and electroencephalographic (EEG) patterns in AGS and look for possible correlations with clinical, genetic and neuroradiological features.

Methods: We selected patients with an established AGS diagnosis followed at three Italian reference centers. Medical records, EEGs and MRI/CT findings were reviewed. EEGs were independently and blindly reviewed by three board-certified pediatric epileptologists. Chi square and Fisher's exact tests were used to test associations between epilepsy and EEG feature categories and clinical, radiological and genetic variables.

Results: Twenty-seven patients were enrolled. We reviewed 63 EEGs and at least one brain MRI scan per patient. Epilepsy, mainly in the form of epileptic spasms and focal seizures, was present in 37 % of the cohort; mean age at epilepsy onset was 9.5 months (range 1–36). The presence of epilepsy was associated with calcification severity ($p = 0.016$) and startle reactions ($p = 0.05$). Organization of EEG electrical activity appeared to be disrupted or markedly disrupted in 73 % of cases. Severe EEG disorganization correlated with microcephaly ($p < 0.001$) and highly abnormal MRI T2-weighted signal intensity in white matter ($p = 0.022$).

Physiological organization of the EEG was found to be better preserved during sleep (87 %) than wakefulness (38 %). Focal slow activity was recorded in more than one third of cases. Fast activity, either diffuse or with frontal location, was more frequent in the awake state (78 %) than in sleep (50 %). Interictal epileptiform discharges (IEDs) were present in 33 % of awake and 45 % of sleep recordings. IEDs during sleep were associated with a higher risk of a epileptic seizures ($p = 0.008$).

Significance: The hallmarks of EEG recordings in AGS were found to be: disruption of electrical organization, the presence of focal slow and fast activity, and the presence of IEDs, both in patients with and in those without epilepsy. The associations between epilepsy and calcification and between EEG pattern and the finding of a highly abnormal white matter T2 signal intensity suggest a common anatomical correlate. However, the complex anatomical-electroclinical basis of AGS-related epilepsy still requires further elucidation.

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1. Introduction

Aicardi-Goutières syndrome (AGS) is a rare hereditary leukodystrophy, named after Jean Aicardi and Françoise Goutières, who first described it in 1984 [1]. To date, causative mutations have been identified in seven genes: TREX1, RNASEH2B, RNASEH2C, RNASEH2A, ADAR1, SAMHD1, IFIH1 [2]. The disease pathophysiology is based on a "fundamental link between nucleic acid metabolism, innate immune sensors and type I interferon induction" [3].

AGS patients with the classic phenotype typically present with a subacute onset during the first year of life. This is characterized by marked irritability, sleep disturbances, feeding difficulties, recurrent sterile pyrexias, progressive loss of previously acquired psychomotor skills, pyramidal and extrapyramidal signs, and slowing of head growth. This initial "encephalitic-like" phase usually lasts some months, and in most cases evolves into spastic-dystonic tetraplegia, typically accompanied by excessive startle reactions and epileptic seizures [4]. The presence of cerebrospinal fluid lymphocytosis and raised levels of interferon-alpha, without evidence of infection, is an important diagnostic feature [2,3]. At the end of the encephalitic-like phase, the active disease seems to "switch off", and there is no clear further progression over time [4,5]. However, signs of abnormal interferon activity (i.e. recurrent chilblains) may persist [2,3].

The classic neuroradiological findings in AGS include cerebral calcification, white matter abnormalities, and brain atrophy [6].

Epileptic seizures have been reported in AGS from the very first description of the syndrome. The incidence of epilepsy in affected patients varies widely in the literature, ranging from 24 % to 75 % of cases [7–13], and remains a matter of debate.

Moreover, although epileptic seizures occur in at least 24 % of patients, their phenotypic and electrophysiological characterization remains elusive.

Ramantani et al., in 2014, [11] made the first (and currently only) attempt to provide a comprehensive evaluation of seizure and EEG features in patients diagnosed with AGS. However, no specific interictal or ictal EEG pattern that might help to distinguish AGS emerged from their study, nor did they identify any discernible correlation between epilepsy, genotype, and neuroimaging findings.

The seizure semiology in AGS includes epileptic spasms, focal tonic and myoclonic, focal to generalized, and generalized tonic-clonic epileptic seizures [8,9,11,14,15]. Electroencephalographic (EEG) tracings characteristically show diffuse slowing of background activity, poor organization of electrical activity, and occasional regional slow-wave activity in interictal recordings 11]. However, in the majority of AGS subjects, EEG has been found to show diffuse slowing without focal or epileptic abnormalities [7,15,16].

This study was conducted to assess epileptic phenotypes, EEG patterns, and their correlations with clinical, genetic, and neuroradiological features in a cohort of AGS patients.

2. Methods

2.1. Protocol approvals and patient consent

The study was approved by our institutional ethics committee (approval n. 3549/2009 of 30/Sep/2009 and 11/Dec/2009, and approval n. 20170035275 of 23/Oct/2017). Informed consent was obtained from all parents or legal guardians of participants.

2.2. Recruitment and inclusion criteria

This is a multicenter study involving AGS patients longitudinally followed at three centers —Mondino Foundation (Pavia), Vittore Buzzi Children's Hospital (Milan), and Spedali Civili (Brescia) — according to a common protocol.

In collaboration with the International Aicardi-Goutières Syndrome Association (IAGSA), we retrospectively evaluated 125 AGS patients followed at the three reference centers from 2000 to 2018. The diagnosis was based on clinical, laboratory, and neuroimaging criteria for AGS [4,5,17], as described by Tonduti and colleagues in 2018 17].

From this cohort, we enrolled patients who met the following inclusion criteria:

- Available clinical records
- At least one digital scalp video-EEG recording
- MRI examination performed within a week of the video-EEG recording.

2.3. Clinical phenotype

We retrospectively collected demographic, genetic, and clinical data as detailed in Table 1.

Symptom onset in relation to age was defined according to the Livingston classification [5]. Data were collected on each clinical diagnostic criterion (i.e., irritability, feeding difficulties, sleeping difficulties, unexplained fevers, chilblains, startle reactions, pyramidal signs, extrapyramidal signs, microcephaly, etc.).

In addition, for the purpose of our study, we evaluated three criteria clinically related to epileptic manifestations:

Global functional impairment. This was recently demonstrated to be a good indicator of global clinical severity [18]. We used a score obtained by summing the scores of the following scales: the Gross Motor Function Classification System [19], the Manual Ability Classification System [20], and the Communication Function Classification System [21]. The resulting composite functional severity score ranged from three (fully preserved motor and communicative function) to 15 (extremely severe impairment of motor and communicative function). A score $\geq$ 12 denoted severe impairment, a score between 6 and 12 moderate impairment, and a score $<$ 6 mild impairment.

- Microcephaly (defined as a head circumference more than two standard deviations below the mean) for age and sex and the related neuroradiological finding of cerebral atrophy. This criterion was taken as an indicator of the severity of cortical neuronal loss and as a factor affecting long-term clinical outcome [20]
- Startle reactions. These are a frequent finding in epileptic encephalopathy patients, which suggests a possible common pathophysiological basis [22].

2.4. Seizures and epilepsy

We reviewed the patients' hospital charts to collect data on their epilepsy history (age at seizure onset and offset, seizure frequency, history of status epilepticus) and use of anti-epileptic drugs (AEDs) (Table 1), as well as details of seizure semiology and of video-EEG recordings. These data were collected and classified according to the 2017 International League Against Epilepsy (ILAE) classification and terminology [23]. In particular, since the seizures were classified mainly retrospectively, on the basis of anamnesis and home videos, the data were then re-evaluated by expert epileptologists, who assigned definitive classifications. We classified seizure semiology as follows: seizures with generalized manifestations (of unknown origin), focal seizures, or spasms (of unknown origin).

2.5. EEG recording, selection, and blinding

Digital scalp video-EEG recordings in wakefulness and during daytime sleep were obtained using a preformed cap with electrodes placed according to the international 10–20 system. A reduced array of electrodes was used for children who had a head circumference of less than 40 cm. All EEGs were performed during the afternoon and lasted a minimum of 1:10 h. Sleep deprivation of at least 50 % of usual sleep time

was required in all patients prior to obtaining daytime sleep recordings.

All identifying information, except for age, was removed before exporting EEG data from digital archives. An alpha-numeric code identified each EEG. Digital videos were available for all subjects.

EEGs were independently and blindly reviewed by two board-certified pediatric electroencephalographers (LG, MV). Any disagreements relating to major rating categories were resolved by a third board-certified pediatric electroencephalographer (V.D.G.).

2.6. EEG rating

EEGs were rated according to the following categories: awake background activity, non-epileptiform interictal abnormalities in wakefulness and sleep, interictal epileptiform discharges (IEDs) in wakefulness and sleep, and ictal recording (including seizure semiology and ictal EEG characteristics). Each major category had sub-descriptors detailing topographical distribution, morphology of EEG patterns and discharges, and correlation with clinical events.

All terminology used was based on standard usage in clinical practice and research. Indeed, we used terms adapted from the standardized computer-based organized reporting of EEG (SCORE) tool [24] and the revised glossary of terms most commonly used by clinical electroencephalographers, proposed by Kane in 2017 [25].

2.7. Neuroimaging

Neuroradiological imaging (MRI and CT) data were reviewed and analyzed only when performed within a week of the video-EEG acquisition.

Two pediatric neurologists with expertise in white matter disorders and AGS imaging analysis (RLP and DT) performed the qualitative imaging review to evaluate the presence, site, and severity of cerebral atrophy, white matter abnormalities, and calcification, according to the approach described in detail by La Piana and colleagues [6].

The presence, site, and severity of calcification were assessed using head CT images. When CT images were not available, calcification was often seen on MRI as areas of high T1-weighted/low T2-weighted signal intensity or low signal intensity on gradient-echo imaging or susceptibility-weighted imaging. Calcification was defined as severe when it involved multiple locations beyond the classical ones — i.e. the lentiform nuclei, deep white matter, thalami — and had multiple and variable patterns, as previously described [26].

MRI images were also reviewed to assess the presence of cortical malformations.

2.8. Statistical analysis

Demographic, clinical, neuroradiological, genetic, and EEG features were analyzed.

Age at disease onset and at epilepsy onset and offset, as well as ages at the time of EEG recordings, were described as mean, range, and/or standard deviation.

Chi-square and Fisher's exact tests were used to test associations between epilepsy and EEG feature categories, and the clinical and neuroradiological variables described above. A p-value ≤ 0.05 was considered statistically significant. We also reported results with a p-value ≤ 0.1, since we cannot exclude that the p-values obtained were dependent on the small sample size. Only results with p-values ≤ 0.05 or ≤ 0.1 are reported in the text.

3. Results

3.1. Population (Table 1)

Our sample included 27 patients: 11 females (41 %) and 16 males (59 %). The EEG recordings in these patients were performed at a mean age of 5.6 years (range 1−21 years; standard deviation 4.7).

- Neurological, seizure and epilepsy features (Table 1)

The onset of AGS occurred within the first year of life in 96 % of the patients (26/27 patients) — in the neonatal period in 33 % (9/27), and between 2 and 12 months of age in 63 % (17/27) —, while in one individual (4%), it occurred at 15 months of age.

Twenty-two patients in our cohort (81 %) showed severe functional impairment, as measured by the composite functional severity score. Microcephaly was observed in 24 of the 27 (89 %), and startle reactions in 19/27 (70 %).

Epilepsy was present in 37 % of the sample (10/27). The mean age at seizure onset was 9.5 months (range 1–36 months): 40 % of the affected patients (4/10) experienced their first seizure in the first three months of life, 40 % (4/10) at between four and 12 months of age, and 20 % (2/10) after the age of 1 year. A younger age at disease onset was more frequent in the patients who showed epilepsy: 100 % of the patients with epilepsy (10/10) had a disease onset before six months of age, versus 59 % (10/17) of those without (p-value: 0.0538). Notably, startle reactions were also present in all the patients with epilepsy (100 % vs 52 %, p-value: 0.050).

Moderate-severe calcification was more frequent in patients with than without epilepsy, being detected in 9/10 and 9/15 respectively (90 % versus 60 %, p-value: 0.016).

With regard to the use of AEDs, mono-therapy effectively controlled seizures in five patients (50 %), whereas polytherapy was necessary in the other five (50 %).

At the time of the study, three patients (mean age 3.6 years; range 3–5) were still affected by seizures, despite ongoing polytherapy, while epilepsy offset and AED discontinuation (in childhood, at a mean age of 7 years; range 2–12) was reported in four. One patient became seizure free in adulthood; another was still affected by seizures at the time of death, which occurred at the age of 4 years, while no information about possible seizure offset was available in one patient.

The following seizure semiology was observed in these 10 AGS patients with epilepsy: seizures with generalized manifestations in two patients, isolated focal motor seizures in two, and epileptic spasms alone in two. A further two children presented initially with epileptic spasms, and subsequently with focal motor seizures; another showed epileptic spasms, followed by seizures with generalized manifestations. Finally, one patient presented with focal motor seizures and seizures with generalized manifestations.

3.2. Neuroradiological findings (Table 2; Fig. 1a-b)

We reviewed neuroradiological imaging data (MRI and CT) from 25 patients (92.6 % of the total cohort).

Brain calcification was observed in 92 % of these patients (23/25): calcification was mild in 44 % (10/23), moderate in 39 % (9/23), severe in 17 % (4/23).

Cerebral atrophy was found in 88 % of the reviewed patients (22/25) and classed as mild in 18 % (4/22), moderate in 59 % (13/22), and severe in 23 % (5/22). Of the 22 patients with cerebral atrophy, 15 had microcephaly, whereas no patient without microcephaly had cerebral atrophy (68 % versus 0%, p-value: 0.0291). Ventricular enlargement was observed in 60 % of the reviewed patients (15/25).

White matter abnormalities were present in all but one patient (96 %; 24/25): these were diffuse in 33 % of cases (8/24), while they showed periventricular predominance in 17 % (4/24) and frontotemporal predominance in 50 % (12/24).

Highly abnormal signal intensity on MRI was observed in 60 % of cases (15/25).

White matter rarefaction located in the frontotemporal poles was observed in 12 % of patients (3/25).

The cerebral cortex was normal in all the subjects. Of note, two

Table 1
Overview of clinical, genetic and epileptological findings in our cohort.

ID	Sex	Genotype	Age at AGS onset	Neurological phenotype	Disease severity score	Head circumference	Presence of startle reactions	Epilepsy	Age at seizure onset	Seizure Type	Seizure frequency	Age at seizure offset	Anti-epilpetic Drugs	Hisotry of febrile seizures	History of status epilepticus
Pt 1	male	AGS5	neonatal	spastic dystonic quadriplegia	>12	<< 3rd p	yes	yes	7 months	epileptic spasms	daily	NA	DZP, VPA	no	no
Pt 2	female	AGS2	2 months	spastic dystonic quadriplegia	>12	<< 3rd p	no	no						no	no
Pt 3	female	AGS2	neonatal	spastic dystonic quadriplegia	>12	< 3rd p	yes	yes	6 months	focal motor	less than monthly	ongoing at 3 years	VPA	no	no
Pt 4	male	AGS3	6 months	spastic dystonic quadriplegia	>12	< 3rd p	yes	no						no	no
Pt 5	female	AGS4	birth	spastic dystonic quadriplegia	>12	<< 3rd p	yes	yes	3 years	generalized tonic-clonic	occasional	10 years	NA	yes	no
Pt 6	male	AGS2	2 months	spastic dystonic quadriplegia	>12	< 3rd p	yes	no						no	no
Pt 7	male	AGS4	birth	spastic dystonic quadriplegia	>12	< 3rd p	yes	yes	3 months	generalized clonic, generalized myoclonic	daily	exitus at 4 years	PHB	no	yes
Pt 8	male	AGS2	neonatal	right hemiplegia	<6	25–50th p	no	no						no	no
Pt 9	male	AGS2	neonatal	spastic dystonic quadriplegia	>12	<< 3rd p	yes	yes	2 months	epileptic spasms, focal motor	daily	ongoing at 5 years	VGB, ACTH, TPM	no	no
Pt 10	female	AGS5	2 months	spastic dystonic quadriplegia	>12	<3rd p	yes	no						no	no
Pt 11	male	AGS2	4 months	spastic dystonic quadriplegia	>12	<3rd p	yes	no						no	no
Pt 12	male	AGS2	2 months	spastic dystonic quadriplegia	>12	<3rd p	yes	yes	6 weeks	generalized tonic-clonic, focal motor	NA	12 years	OXC	no	yes
Pt 13	female	AGS2	2 months	spastic dystonic quadriplegia	>12	<3rd p	yes	yes	3 months	epileptic spasms, generalized tonic	NA	27 years	NZP, HC, CZP, LTG	no	no
Pt 14	female	UNK	4 months	spastic dystonic quadriplegia	>12	<3rd p	yes	yes	5 months	epileptic spasms, focal motor	daily	2 years	VGV, CZP, TPM, RFN	no	no
Pt 15	male	AGS1	birth	spastic dystonic quadriplegia	>12	< 3rd p	yes	no						no	no
Pt 16	female	AGS2	3 months	dyplegia plus left arm	>12	3rd-10th p	yes	no						no	no
Pt 17	male	AGS2	2 months	spastic dystonic quadriplegia	>12	< 3rd p	yes	yes	2 years	focal motor	NA	4 years	VPA	yes	no

(*continued on next page*)

Table 1 (*continued*)

ID	Sex	Genotype	Age at AGS onset	Neurological phenotype	Disease severity score	Head circumference	Presence of startle reactions	Epilepsy	Age at seizure onset	Seizure Type	Seizure frequency	Age at seizure offset	Anti-epilpetic Drugs	Hisotry of febrile seizures	History of status epilepticus
Pt 18	female	AGS2	15 months	spastic dyplegia	<6	75−90th p	no	no						no	no
Pt 19	female	AGS2	9 months	spastic dystonic quadriplegia	>12	< 3rd p	no	no						no	no
Pt 20	male	AGS2	neonatal	spastic dyplegia	<6	< 3rd p	no	no						no	no
Pt 21	male	AGS2	11 months	spastic dystonic quadriplegia	>12	<3rd p	yes	no						no	no
Pt 22	male	AGS2	6 months	spastic dystonic quadriplegia	>12	< 3rd p	yes	no						no	no
Pt 23	male	AGS6	7 months	spastic dystonic quadriplegia	>12	<< 3rd p	no	no						no	no
Pt 24	male	AGS5	4 months	spastic dystonic quadriplegia	>12	<3rd p	yes	yes	8 months	epileptic spasms	daily	ongoing at 4 years	HC, VGB, LTG	no	no
Pt 25	female	UNK	1 month	spastic dystonic quadriplegia	<6	< 3rd p	yes	no						no	no
Pt 26	male	AGS2	11 months	spastic diplegia	>6 < 12	97th p	no	no						no	no
Pt 27	female	AGS2	5 months	axial hypotonia	>12	< 3rd p	no	no						no	no

Abbreviations: Ptpatient; ppercentile; NAdata not available; DZPdiazepam, VPA, valproic acid; PHBphenobarbital; VGBvigabatrin; ACTHadrenocorticotropic hormone; TPMtopiramate; OXCox-carbamazepine; NZPnitrazepam; HChydrocortisone; CZPclonazepam, LTG, lamotrigine; RFNrufinamide.

Table 2
Overview of neuroradiological findings in our cohort.

PATIENT	leukoencephalopathy presence	symmetrical distribution	highly abnormal signal intensity	predominance of white matter involvement	atrophy severity	ventricular enlargement	cerebral cortex	calcification presence
Pt 1	present	yes	present	antero-posterior gradient	severe	present	normal	severe
Pt 2	present	yes	absent	antero-posterior gradient	moderate	absent	normal	mild
Pt 3	present	yes	present	antero-posterior gradient	severe	absent	normal	severe
Pt 4	present	yes	present	antero-posterior gradient	moderate	present	normal	mild
Pt 5	present		present	antero-posterior gradient	moderate	present	normal	severe
Pt 6	present	yes	present	antero-posterior gradient	mild	present	normal	moderate
Pt 7	present	yes	present	periventricular	moderate	present	normal	severe
Pt 8	present	yes	absent	diffuse	absent	absent	normal	moderate
Pt 9	present	yes	present	antero-posterior gradient	moderate	present	abnormal with calcification	moderate
Pt 10	NA	NA	NA	NA	NA	NA	NA	NA
Pt 11	NA	NA	NA	NA	NA	NA	NA	NA
Pt 12	present	yes	present	antero-posterior gradient	moderate	present	normal	moderate
Pt 13	present	yes	absent	antero-posterior gradient	moderate	absent	normal	moderate
Pt 14	present	yes	absent	diffuse	mild	present	abnormal, thin	mild
Pt 15	present	yes	present	antero-posterior gradient	severe	absent	normal	moderate
Pt 16	present	yes	absent	periventricular	absent	absent	normal	mild
Pt 17	present	yes	present	antero-posterior gradient	severe	present	normal	mild
Pt 18	present	yes	absent	periventricular	moderate	absent	normal	mild
Pt 19	present	yes	present	antero-posterior gradient	mild	absent	normal	moderate
Pt 20	present	yes	absent	antero-posterior gradient	moderate	present	normal	mild
Pt 21	present	yes	present	periventricular	moderate	present	normal	absent
Pt 22	present	yes	present	diffuse	severe	present	normal	moderate
Pt 23	present	yes	present	periventricular	moderate	present	normal	moderate
Pt 24	present	yes	present	diffuse	moderate	present	abnormal, thin	mild
Pt 25	present	yes	absent	periventricular	moderate	present	normal	mild
Pt 26	absent		absent		absent	absent	normal	absent
Pt 27	present	yes	absent	diffuse	mild	absent	normal	mild

Abbreviations: NA, data not available.

patients presented specific cortical atrophy, and one showed cortical calcification.

3.3. EEG features (Table 3; Fig. 2)

We reviewed 63 EEGs from 27 patients. Two EEG recordings were of suboptimal quality and could be evaluated only for background activity. We had at least one evaluable EEG recording per patient, and in 55 % of the cohort (15/27 cases) more than one. On average, 2.3 recordings were evaluated per patient (range 1–7, standard deviation 1.6), with a mean of five years (standard deviation 4.4) separating the first and last EEG.

Background activity analysis showed age-appropriate dominant posterior activity in four individuals (15 % of the cohort), a slower dominant posterior rhythm in 11 (41 %), and diffuse delta activity in 12 (44 %). A significant association was found between slowing of EEG background activity and highly abnormal signal intensity on MRI (p-value: 0.028). Normal organization of electrical activity on EEG was found in four individuals (15 % of the cohort), while three (12 %) showed discrete organization; conversely, disorganized and markedly disorganized electrical activity was found in 16 (58 %) and 4 (15 %) cases, respectively. These 20 cases with disorganized or markedly disorganized electrical activity on awake EEG accounted for the majority (83 %) of the 24 patients with microcephaly, while the other microcephalic AGS patients showed normal or discrete organization (p-value <0.0001). Moreover, disorganized electrical activity on awake EEG was also more frequent in the patients with versus without highly abnormal signal intensity on MRI (70 % versus 21 %, p-value: 0.022). The patients with an early disease onset (<6 months of age) were more likely to show marked disorganization of awake EEG activity compared with the late-onset patients (17/20, 80 % versus 57 %, p-value: 0.075). Marked EEG awake disorganization was also found to be more frequent in the patients with greater disease severity (score $\geq$12) than in those with lower disease severity scores (16/22, 77 % versus 40 %, p-value: 0.059). Interhemispheric asymmetry was present in 15 % of the cohort (4/27). Normal sleep waves were detectable in 50 % of the available sleep EEG recordings (11/ 22).

Focal slow activity during wake or sleep EEG was seen in 33 % of the cohort (9/27). Specifically, focal slow activity in awake recordings was found 15 % of the cases (4/27), whereas it was present in 32 % of the available sleep EEG recordings (7/22 recordings). In the awake recordings, it was frontal in two cases, central in one, and occipital in the other, whereas in the seven sleep recordings, it was centrally located in four recordings, and located in frontal, temporal, and occipital regions in one recording each.

Fast electrical activity was present in 78 % of the cohort (21/27) and in 50 % of sleep recordings (11/22). In the awake recordings, it was generalized in 13 of cases (62 %), involved frontal regions in six (28 %), and was observed in posterior regions in two (10 %), whereas in the sleep recordings, it was generalized in six (55 %) and located in the frontal lobes in the other five (45 %). The use of drugs potentially able to influence electrical activity speed was excluded in all individuals.

Interictal epileptiform discharges (represented by spikes/polyspikes, sharp/polysharp waves, spike and slow-wave complexes, and sharp and slow-wave complexes) were present in 33 % (9/27) of patients. They were generalized in 22 % (2/9), located in the temporal lobes in 66 % (6/9), and observed in the frontal lobes in 11 % (1/9). IEDs were present during sleep in 45 % of available recordings (10/22), showing a generalized distribution in 30 % and a focal distribution in 70 % (being detected in central areas in 30 % and in temporal areas in 40 %). IEDs during sleep were more frequent in patients with epileptic seizures (71 %) than in those without (33 %) (p-value 0.008).

No hypsarrhythmia pattern was recorded in any recording.

Ictal EEGs were recorded in two cases. In one, the event manifested itself clinically as a cluster of spasms with corresponding electrical activity compatible with diffuse slow-wave complexes with fast activity. The other event presented as a focal motor seizure associated with electrical activity compatible with focal epileptiform discharge located

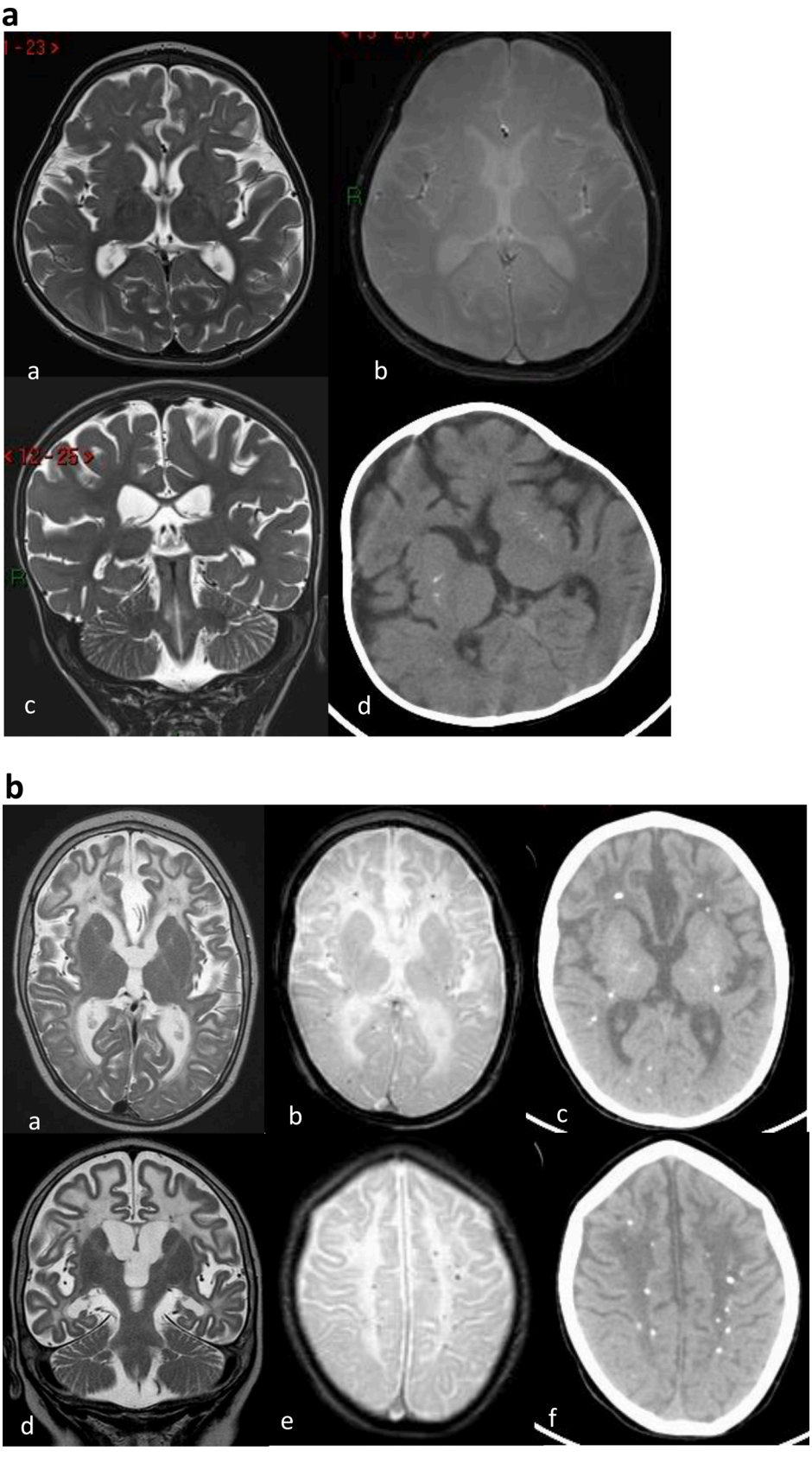

Fig. 1. a. Patient #20 (mild course). Axial and coronal T2 TSE (a,c) and T2 GE (b) MRI sequences and axial CT (d) of the same patient #20, showing mild cerebral atrophy with no white matter abnormalities (a,c) and the presence of concomitant bilateral basal ganglia calcifications (b,d). b. Patient #9 (severe course).
Axial T2 TSE (a,d) and T2 GE (b,e) MRI images and axial CT (c,f) of the same patient #9. The figure shows diffuse white matter abnormalities, detectable as highly abnormal signal intensity on T2-weighted images, associated with global enlargement of the ventricular system and mild cortical atrophy (a,b,d,e). CT images (c,f) show diffuse scattered calcification evident in both basal ganglia, in the thalami, in the hemispheric white matter bilaterally and the cortical-juxtacortical junction mainly on the fronto-parietal left side (f); the same calcification is are evident as multiple focal hypointensities on T2 GE images (b,e)

Table 3
Overview of electroencephalographic findings in wake and sleep in our cohort.

PATIENT	BACKROUND ACTIVITY		INTERICTAL WAKE						ICTAL (CLINICAL)	ICTAL (EEG)	DROWSINESS AND SLEEP							INVOLUNTARY MOVEMENTS
	FREQUENCY	ORGANIZATION	FOCAL SLOW ACTIVITY	FAST ACTIVITY	EPILEPTIFORM DISCHARGES						FOCAL SLOW ACTIVITY	FAST ACTIVITY	EPILEPTIFORM DISCHARGES				physiological sleep waves	
					SPIKE WAVE / POLYSPIKES	SHARP / POLYSHARPS	SPIKE AND SLOW-WAVE COMPLEX	SHARP AND SLOW-WAVE COMPLEX					SPIKE WAVE / POLYSPIKES	SHARP / POLYSHARPS	SPIKE AND SLOW-WAVE COMPLEX	SHARP AND SLOW-WAVE COMPLEX		
Pt 1	slowing posterior rythhm	disorganized	FCP, bilateral	FCP, bilateral	FCP, bilateral	FCP, bilateral	generalized	FCP, bilateral	spasms	generalized	FCP, bilateral	FCP, bilateral	FCP, bilateral	absent	FCP, bilateral	absent	present	present
Pt 2	diffuse delta activity	markedly disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	present
Pt 3	slowing posterior rythhm	disorganized	absent	F, bilateral	absent	absent	absent	absent	absent	absent	PO, bilateral	F, bilateral	O, bilateral	absent	absent	absent	present	absent
Pt 4	diffuse delta activity	markedly disorganized	FP, bilateral	generalized	absent	absent	absent	generalized	absent	absent	absent	absent	absent	absent	absent	generalized	absent	present
Pt 5	slowing posterior rythhm	disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	present
Pt 6	diffuse delta activity	disorganized	absent	F, right	absent	absent	absent	absent	absent	absent	absent	F, bilateral	absent	absent	generalized	absent	absent	present
Pt 7	slowing posterior rythhm	disorganized	absent	absent	absent	T, bilateral	absent	absent	absent	absent	absent	absent	absent	T, bilateral	absent	absent	absent	present
Pt 8	adequate for age	normal	absent	FC, bilateral	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
Pt 9	diffuse delta activity	markedly disorganized	absent	PT, bilateral	absent	T, left	absent	absent	focal seizure	FT, bilateral	absent	PT, bilateral	absent	absent	absent	absent	absent	present
Pt 10	diffuse delta activity	disorganized	absent	absent	absent	absent	absent	absent	absent	absent	CT, right	absent	absent	absent	absent	absent	present	absent
Pt 11	slowing posterior rythhm	discrete organization	absent	generalized	absent	absent	absent	absent	absent	absent	CP, right	generalized	absent	absent	absent	absent	present	absent
Pt 12	diffuse delta activity	markedly disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	absent	absent	absent	FCT, bilateral	absent	absent	absent	present
Pt 13	adequate for age	normal	absent	absent	absent	PT, bilateral	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	absent
Pt 14	diffuse delta activity	disorganized	absent	FC, bilateral	absent	absent	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	absent
Pt 15	diffuse delta activity	disorganized	absent	FT, bilateral	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	present	absent
Pt 16	slowing posterior rythhm	discrete organization	absent	TPO, bilateral	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	present	absent
Pt 17	slowing posterior rythhm	disorganized	absent	generalized	absent	O, bilateral	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	present	absent
Pt 18	adequate for age	normal	absent	absent	absent	absent	absent	absent	absent	absent	CTO, bilateral	absent	absent	absent	generalized	absent	present	absent

(*continued on next page*)

Table 3 (*continued*)

PATIENT	BACKROUND ACTIVITY		INTERICTAL WAKE						ICTAL (CLINICAL)	ICTAL (EEG)	DROWSINESS AND SLEEP							INVOLUNTARY MOVEMENTS
	FREQUENCY	ORGANIZATION	FOCAL SLOW ACTIVITY	FAST ACTIVITY	EPILEPTIFORM DISCHARGES						FOCAL SLOW ACTIVITY	FAST ACTIVITY	EPILEPTIFORM DISCHARGES				physiological sleep waves	
					SPIKE WAVE / POLYSPIKES	SHARP / POLYSHARPS	SPIKE AND SLOW-WAVE COMPLEX	SHARP AND SLOW-WAVE COMPLEX					SPIKE WAVE / POLYSPIKES	SHARP / POLYSHARPS	SPIKE AND SLOW-WAVE COMPLEX	SHARP AND SLOW-WAVE COMPLEX		
Pt 19	diffuse delta activity	disorganized	absent	generalized	absent	FCT, right	absent	absent	absent	absent	absent	FT, bilateral	absent	absent	FCT, bilateral	absent	absent	present
Pt 20	slowing posterior rythhm	disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	CT, right	generalized	absent	absent	absent	absent	present	absent
Pt 21	slowing posterior rythhm	discrete organization	T, bilateral	generalized	absent	absent	absent	absent	absent	absent	T, bilateral	a	absent	absent	absent	absent	present	absent
Pt 22	slowing posterior rythhm	disorganized	absent	absent	absent	absent	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	absent
Pt 23	diffuse delta activity	disorganized	absent	generalized	absent	absent	absent	TO, bilateral	absent	absent	absent	generalized	absent	absent	absent	TO, bilateral	absent	absent
Pt 24	diffuse delta activity	disorganized	absent	generalized	absent	absent	absent	TO, bilateral	absent	absent	absent	generalized	absent	absent	absent	TO, bilateral	absent	absent
Pt 25	diffuse delta activity	disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	absent	generalized	absent	absent	absent	absent	absent	present
Pt 26	adequate for age	normal	absent	F, bilateral	absent	absent	absent	absent	absent	absent	absent	F, bilateral	absent	absent	absent	absent	present	absent
Pt 27	slowing posterior rythhm	disorganized	C, bilateral	absent	absent	absent	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	absent

Abbreviations: Pt, patient; F, frontal; C, central; P, parietal; T temporal; O, occipital; NA, data not available.

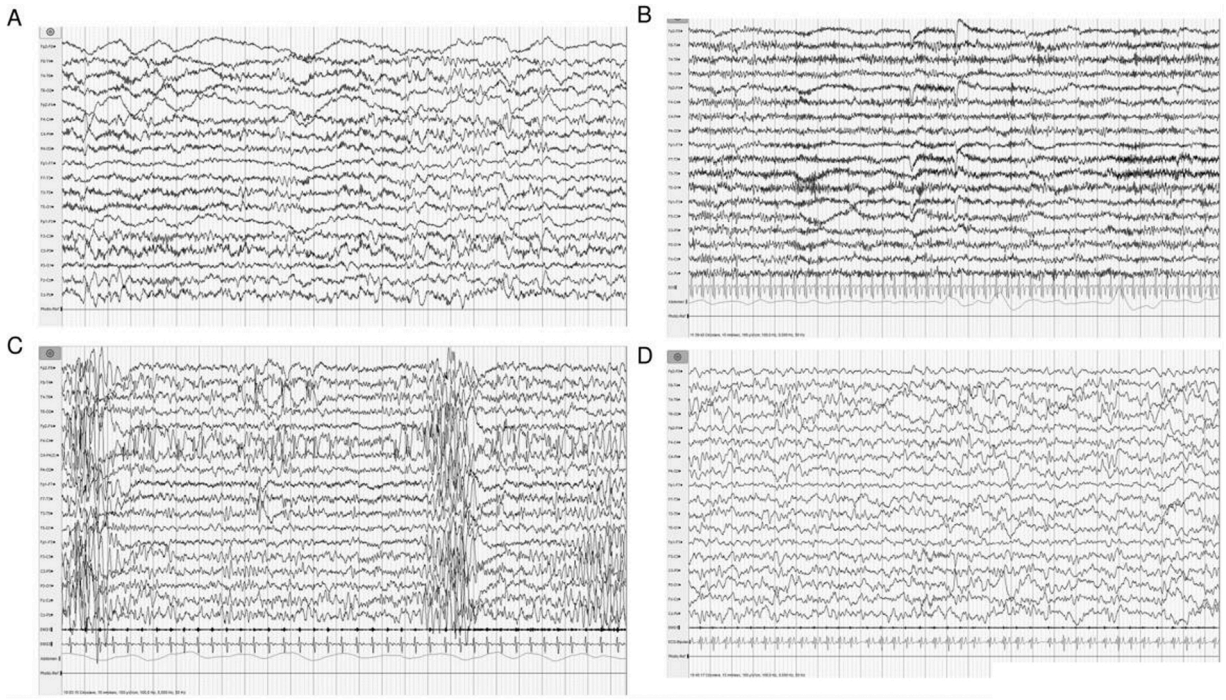

Fig. 2. A. focal fast activity in the fronto-central region in an awake recording; B. diffuse fast activity in an awake recording; C. generalized interictal epileptiform discharges, prevalently in fronto-temporo-parietal regions; D. focal slow activity in temporo-posterior regions in a sleep recording.

in the frontotemporal regions bilaterally.

The video-EEG recordings captured startle reactions, provoked by noise or spontaneous, but these were never associated with EEG modifications (Fig. 3).

4. Discussion

Our study provides a detailed description of EEG features and their relationships with clinical and neuroradiological data in AGS. Only one previous study sought to furnish a comprehensive evaluation of seizure and EEG features in patients with AGS [11]. However, Ramantani et al. did not investigate the presence of specific interictal or ictal EEG patterns in AGS patients, and, due to their small sample size, they did not perform any correlation analysis between epilepsy, genotype, and neuroimaging findings.

The clinical and radiological features and genotypes of our sample of AGS patients confirm what has already been reported in more extensive series [1,2,6,9,13,17,26–28].

Epilepsy was found in approximately 40 % of our cohort, a rate similar to that reported by Crow [2], but lower than that found by Ramantani et al. [11]. This difference might be due to a possible selection bias in Ramantani's cohort, which was recruited in an epilepsy centre; our patients, on the other hand, were followed at third-level institutes (all three are Italian and international referral centers for AGS).

In 80 % of our sample, epilepsy onset occurred within the first year of life and its presence was statistically significantly correlated with an early disease onset.

Since this was a retrospective study, the seizure semiology had to be established on the basis of anamnestic dataoccasionally supported by patients' home videos. It consisted of epileptic spasms and focal motor seizures in equal measure and, to a slightly less extent, seizures with generalized manifestations.

Although our study did not set out to assess the efficacy of AEDs in AGS patients, our data suggest that in at least 50 % of cases epilepsy is easily controlled with the first AED administered, and that more than 50 % of these patients become seizure free before adolescence.

While acknowledging the limitations due to the small size of our sample, we consider it useful to underline that vigabatrin was the drug of choice for spasms.

In 70 % of patients, we observed startle reactions, and these were statistically significantly correlated with the presence of epilepsy. Startle reactions are a physiological motor response to sudden sensitive stimuli; in some pathological conditions they can be provoked by even mild stimuli and are not susceptible to habituation effects. The bulbopontine reticular formation is the anatomical generator of the startle reaction [22]. The precise pathophysiology of the exaggerated startle reaction observed in AGS is still not clear. However, its higher incidence in AGS patients with epilepsy may point to the presence, in AGS, of a neuronal dysfunction involving both cortical and reticular neurons and/or their interconnection.

Our analysis of EEG activity revealed that physiological EEG organization was better preserved in sleep than in awake recordings. Electrical activity showed moderate to marked disorganization in 70 % of our subjects, and unsurprisingly this finding was more frequent in patients with a higher disease severity score. EEG organization did not differ between patients with and without epilepsy, an observation that reflects the fact that AGS is not an epileptic encephalopathy in the strict sense, rather an encephalopathy that can be associated with epileptic seizures. Severe EEG disorganization was statistically significantly correlated with microcephaly, which in turn was found to be directly associated with an increased likelihood of cerebral atrophy. Therefore, we can infer that marked EEG disorganization correlates with atrophy. As reported in previous papers [6,26], atrophy in AGS is due to white matter loss rather than involvement of the primary cortex, which suggests that brain white matter may play a role in EEG organization. This hypothesis is further supported by the finding of a correlation between highly abnormal white matter T2-weighted signal intensity on MRI and moderate-severe EEG disorganization.

A peculiar low-amplitude fast activity at about 12−16 Hz, previously described in just one AGS patient [1], was a frequent finding in our cohort's awake EEGs. EEG fast rhythms are known to be a typical finding

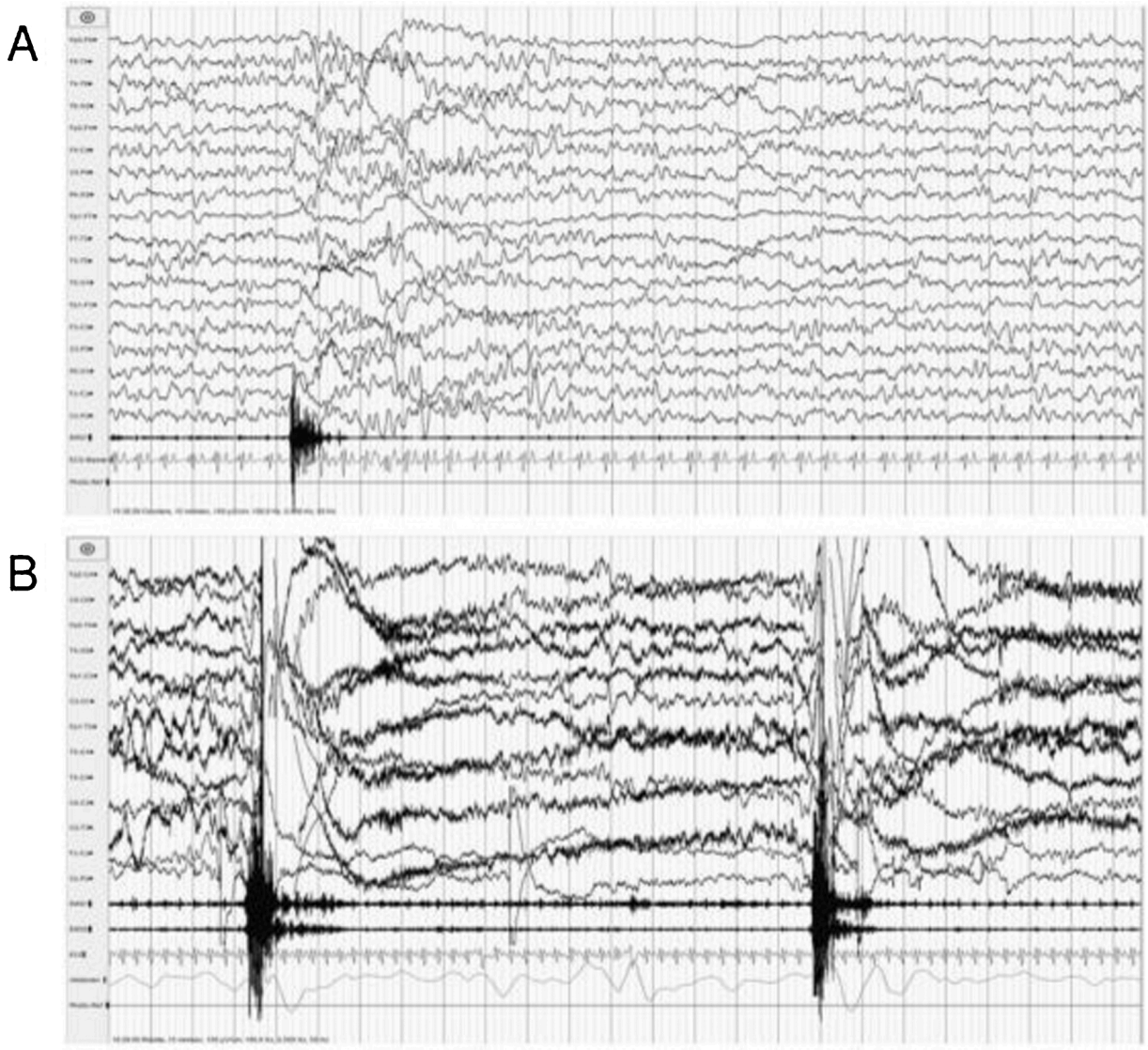

Fig. 3. A. startle reaction, provoked by noise, with no EEG modifications; B. epileptic spasms with symmetrical limb flexion, preceded by generalized slow wave with superimposed fast activity.

in lissencephaly [29], and have also been described as non-specific finding in other brain malformations [30,31]. We did not observe any cortical malformations in our cohort. These observations, taken together with other reports in non-malformative genetic syndromes [32], suggest that increased fast activity may reflect the presence of cortical and subcortical abnormalities or dysfunctional circuitries caused by microscopic cortical disruption. Future in-depth studies are needed to identify a possible pathogenic mechanism underlying this pattern.

Focal slow activity was present in more than one third of patients, more frequently in sleep recordings, as previously described by Ramantani et al. [11].

Interictal epileptiform discharges were present in one third of wake EEG recordings and almost half of sleep recordings. We observed polyspikes, sharp waves, and sharp slow waves. In our sample, IEDs more often showed a focal than a generalized location. Topographically, focal IEDs were more commonly seen in central and temporal regions, similarly to what was reported by Ramantani et al. [11]. As expected, IEDs in sleep recordings occurred more frequently in patients with a diagnosis of epilepsy.

In 62 % of the cases with focal EEG activity, this activity corresponded to a localized neuroradiological characteristic (such as a particular pattern of predominance of white matter involvement, calcification or cystic degeneration), while in the remaining patients, focal EEG abnormalities did not correlate with focal neuroradiological findings. This complex anatomical-electroclinical picture needs to be further explored in future studies.

Overall, the pathogenesis of epileptic seizures and EEG abnormalities in AGS requires further eludication. With the exception of “astrocytopathies” [33,34], epilepsy is rarely documented in the early stages of leukodystrophies. Astrocytes have many essential functions, including brain ion and water homeostasis, and their dysfunction can trigger seizures [35,36]. Moreover, astrocytes are key players in neuroinflammation, which plays a central role in the pathogenesis of AGS [37]. Both innate and adaptive immunity are involved in seizure genesis. The authors of a fairly recent study [38] comparing epileptic patients with healthy subjects suggested that postictal and interictal inflammatory cytokines (IL-6, IL-1 beta, IL-10, IL-17A, TNF-α, and IFN-γ) are elevated in the plasma of active epilepsy patients. In most of our

patients, epilepsy appeared early in the disease course, specifically in the "encephalitic phase" that is related to high interferon levels. Thus, interferon may play a role in both disease and epilepsy onset in AGS. Should further studies confirm this hypothesis, it would have important therapeutic implications for the management of patients with interferonopathies.

Of note, in our cohort, seizure burden was directly correlated with calcification severity, a finding that could indicate a causal relationship between the two parameters, or point to a common pathogenic mechanism. In recent decades, there has been a growing interest in the role of cerebral calcification in the pathogenesis of epileptic seizures [39–41] and in disorders with a primary extraneurological phenotype associated with seizures [42,43].

In conclusion, this is the first study conducted to provide a detailed description of EEG and epileptic seizure features in AGS and to explore their possible correlations with clinical and neuroradiological data. The hallmarks of EEG recordings in AGS were found to be: disruption of electrical organization, the presence of focal slow and fast activity, and the presence of IEDs, both in patients with and in those without epilepsy.

The mechanism underlying the development of epilepsy in AGS remains to be clarified. Taken together, various aspects — the severity of the cerebral abnormalities, the presence of cortical calcification, the possible roles of astrocytic dysfunction and of neuroinflammation, and the hypothetical involvement of neurons and neuronal circuits of the cerebral cortex and reticular formation — support the argument for a multifactorial basis.

Our study was conducted in a limited number of patients. Further studies in larger samples will be needed to confirm our results.

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Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Declaration of Competing Interest

Dr. De Giorgis received a consultancy fee from Nutricia Gmbh. Dr. Varesio received a consultancy fee from the Italian GLUT1 association (a no-profit organization). Prof. Veggiotti received financial support (speaker fee, travel expenses) from Nutricia GmbH, Dr. Schär AG/SPA, Eisai. None of the other authors has conflict of interest to disclosure.

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Appendix A

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