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# Title: "Phenotypic spectrum and prognostic factors in epilepsy with eyelid myoclonia"

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**Abbreviations:**

ADHD = attention deficit hyperactivity disorder ; AEO = age at epilepsy onset ; ASM = antiseizure medication ; body-MYO = sporadic myoclonia over body districts other than eyelids ; AUC = area under the curve ; CAE = childhood absence epilepsy ; CNV = copy number variant ; DE = developmental encephalopathy ; DEE = developmental and epileptic encephalopathy ; ECS = eye closure sensitivity ; EE = epileptic encephalopathy ; EEM = epilepsy with eyelid myoclonia ; EEG = electroencephalography ; EM = eyelid myoclonia ; FS = febrile seizure ; GABA = gamma-ammino-butyric-acid ; HR = hazard ration ; ID = intellectual disability ; ILAE = international league against epilepsy ; GGE = genetic generalized epilepsy ; GPFA = generalized paroxysmal fast activity ; GTCA= generalized tonic-clonic seizure alone syndrome ; GTCS = generalized tonic-clonic seizures ; IGE = idiopathic generalized epilepsy ; IQR = interquartile range ; JAE = juvenile absence epilepsy ; JME = juvenile myoclonic epilepsy ; KDE = kernel density estimation ; LEV = levetiracetam ; LTG = lamotrigine ; MRI = magnetic resonance imaging ; OR = odd ratio ; PS = photosensitivity ; PWD = polyspike-wave discharge ; SD = standard deviation ; STR = sustained terminal remission ; SWD = spike-wave discharge; TSCA = two-step cluster analysis ; VPA = valproate.

## 1. Abstract

Genetic generalized epilepsy (GGE) represents a common form of epilepsy both in adult and children's cohorts. The International League Against Epilepsy recently provided a new classification framework distinguishing idiopathic generalized epilepsy (IGE) syndromes from other non-IGE syndromes within the context of GGE, in view of the strong overlap in terms of genetics, electroclinical features, and prognosis observed in the former group. If on the one hand previous studies thoroughly delineated the clinical characteristics of IGE, on the other still much work is needed to better define other non-IGE syndromes. Epilepsy with eyelid myoclonia (EEM) represents one of the most common non-IGE syndromes encountered in clinical practice and has been characterized by the classical triad of eyelid myoclonia with or without absences, photosensitivity, and eye closure sensitivity. Previous studies focusing on EEM have been conducted on small cohorts of patients revealing a marked clinical heterogeneity.

In the present thesis, we will first provide an updated overview of the nosology and the electroclinical features of GGE syndromes, mainly focusing on EEM and discussing the major limitations of existing literature. Next, in the experimental part of the thesis, we will illustrate the results from three original studies conducted on the largest cohort of patients ever recruited so far. Indeed, during the past three years, we had the opportunity to coordinate an international study group including 20 epilepsy centers, which allowed us to enroll 267 EEM patients. We investigated the electroclinical features and the long-term seizure outcome of EEM, highlighting the existence of homogenous disease sub-phenotypes through the use of modern clustering techniques, and we underscored relevant clinical and prognostic differences based on sex. Finally, we will provide a unifying interpretation of our results, which support the hypothesis of EEM as a spectrum disorder, and we will discuss their implication in epilepsy care and research.

## 2. Introduction

### *2.1 Genetic generalized epilepsy: general clinical features and classification*

Genetic generalized epilepsy (GGE) encompasses several epilepsy syndromes with a strong genetic background (Mullen and Berkovic, 2018). A high familial aggregation has been observed, with relatives of patients with GGE having an 8.3-fold increased risk of developing GGE when compared with the general population (Peljto et al., 2014). However, very few cases of GGE are explained by simple mendelian inheritance and most have a genetically complex pattern of inheritance with modest penetrance (Marini et al., 2004). A polygenic basis, with or without a contribution from environmental factors, has been therefore hypothesized in the majority of cases, although in some patient's monogenic disease genes have been increasingly identified (Nicita et al., 2012; Kang et al., 2016; Suzuki et al., 2004). Not in accordance with epilepsy in general (which has been described slightly more prevalent in men), the incidence and prevalence of GGE is higher in women than in men (Videira et al., 2021; Christensen et al., 2005), and the general frequency has been estimated at 15–20% of all epilepsies in adult and children's cohorts (Jallon et al., 2005).

GGE patients may experience one or a combination of the following generalized seizure types: absences, myoclonic, tonic–clonic, and myoclonic–tonic–clonic seizures (Fisher et al. 2017; Hirsch et al., 2022). The electroencephalography (EEG) hallmark of GGE are bilateral synchronous, symmetrical, and generalized spike-wave discharges (SWD), polyspikes and polyspike-wave discharges (PWD), typically activated by NREM sleep and awakening (Seneviratne et al., 2017; Betting et al., 2006). The occurrence of these highly stereotyped EEG patterns has been explained by the existence of hypersynchronous neural activity patterns within interconnected circuits between the

thalamus and the cortex (Avoli et al., 2012; Beenhakker and Huguenard, 2009), and after many decades, a unifying theory on the generation of these discharges has been proposed. The initial event has been hypothesized to be the generation of a normal or epileptic spike at the site of the cortical focus (which may anatomically differ according to different GGE sub-syndromes), rapidly propagating through bilateral cortico-thalamic networks that form a resonant circuitry in which the thalamus and the cortex drive each other (Meeren et al., 2005; McCafferty et al., 2018). Similarly, generalized onset seizures have been defined as seizures "originating at some point within, and rapidly engaging, bilaterally distributed networks", in spite of the possible origin at the site of a cortical focus (Fischer et al., 2017).

The combination of generalized seizure types observed, along with the age at epilepsy onset and other electroclinical features helps to define the different sub-syndromes within the context of GGE. The International League Against Epilepsy recently provided a new classification framework for GGE syndromes, in which they distinguished idiopathic generalized epilepsies (IGE) from other non-IGE syndromes (Hirsch et al., 2022).

*2.1. Idiopathic generalized epilepsies (IGEs)*

The ILAE included among IGEs the following syndromes: childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME) and generalized tonic-clonic seizures alone (GTCA). These four syndromes were considered as a distinct subgroup of the GGEs, due to their generally good prognosis and their strong clinical and genetic overlap (Cerulli Irelli et al., 2020a, Gesche et al., 2022).

IGEs follow complex inheritance, where they arise due to a polygenic basis with or without an environmental contribution. However, in a small proportion of IGE patients, monogenic causes have been identified, including several gamma-aminobutyric acid

(GABA) receptor subunit genes (e.g., GABRG2, GABRA1) (Wallace et al., 2001; Cossette et al., 2002), the gene encoding glucose transporter 1 (SLC2A1) (Arsov et al., 2012), and other rare genes (e.g. EFCH1, CLCN2, etc.) (Cossette, 2010; Suzuki et al., 2004). Recurrent copy number variants (CNVs), such as microdeletions and microduplications, could occur in 3% of patients with IGE (Helbig et al., 2009; de Kovel et al., 2010), and they are likely to be one of the polygenic factors that contribute to the etiology of these disorders, rather than be wholly causative (Dibbens et al., 2009). These CNVs can be familial or arise de novo, and substantially increase the risk of IGE (Dibbens et al., 2009).

IGE syndromes differ in their age of onset, which typically ranges from 3 to 25 years (Hirsch et al., 2022). Although response to antiseizure medications (ASMs) and need for long-term therapy vary within individual syndromes, the IGE syndromes are usually drug responsive, with about 80% of patients responding to appropriate antiseizure medications (Jallon et al., 2005; Seneviratne et al., 2012). For generalized tonic–clonic seizures, valproate (VPA) may be particularly efficacious but should be used with caution in women of childbearing age (Nicolson et al., 2004; Cerulli Irelli et al., 2020a). Other ASMs could also be used in the treatment of GGEs, namely, ethosuximide (particularly effective in the treatment of absences), benzodiazepines (especially clobazam and clonazepam), levetiracetam, lamotrigine, zonisamide, topiramate, and barbiturates (Kanner and Bicchi, 2022). Importantly, certain ASMs, particularly sodium channel blockers, including carbamazepine, oxcarbazepine, eslicarbazepine, and phenytoin (but not necessarily lamotrigine), and GABAergic agents, such as tiagabine and vigabatrin, often exacerbate absence and myoclonic seizures in IGE (and may even provoke absence or myoclonic status epilepticus), providing a clue to diagnosis (Chaves et al., 2005; Cerulli Irelli et al., 2021).

In spite of this generally good long-term prognosis, the IGE syndromes differ in their likelihood to remit and the age of remission, and patients may sometimes evolve from one IGE syndrome to another (typically CAE patients may evolve into JME in adolescence) (Seneviratne et al., 2012). Patients with IGE will experience one or a combination of the following generalized seizure types: absence, myoclonic, tonic–clonic, and myoclonic–tonic–clonic seizures. The occurrence of tonic, atonic, myoclonic–atonic, focal seizures and epileptic spasms exclude a diagnosis of IGE (Hirsch et al., 2022). Seizures occur more often during the first hours after awakening (especially in JME and GTCA) and are usually triggered by sleep deprivation and alcohol consumption.

A photoparoxysmal response occurs with intermittent photic stimulation in most untreated patients with JME and a minority of patients with CAE and JAE (Fisher et al., 2022). A normal routine EEG does not exclude a diagnosis of IGE in the setting of convincing clinical evidence (i.e., a good description of myoclonic seizures with appropriate age at onset). In such cases, a sleep-deprived or prolonged EEG recording may elicit generalized spike-wave discharges (Smith et al., 2005).

Although intellect is almost invariably normal in IGE, mood disorders, anxiety, attention-deficit/hyperactivity disorder (ADHD), and learning disorders have been described (Hirsch et al., 2022). Importantly, the IGEs have also been correlated with poorer long-term social outcomes, including decreased academic achievement; increased risk of unplanned pregnancy; psychiatric, emotional, and behavior problems; and decreased social interaction with friends (Gesche et al., 2021).

The main clinical and EEG characteristics of all IGE syndrome are summarized in Table 1.

*2.2 Non-IGE genetic generalized epilepsies*

In addition to the IGEs, other GGE syndromes included in the latest classification proposals were: 1) individuals with generalized seizure types and generalized SWD and PWD who do not meet criteria for a specific IGE syndrome; and 2) other less common and less defined GGE syndromes.

Among the latter, the ILAE recognized the following entities: myoclonic epilepsy in infancy, epilepsy with myoclonic absences, epilepsy with myoclonic-atonic seizures, and epilepsy with eyelid myoclonia (EEM) (Specchio et al., 2022). These syndromes also have a genetic basis and may occur in the setting of normal intellect or intellectual disability.

Some syndromes, such as epilepsy with myoclonic-atonic seizures, may present with an epileptic encephalopathy (i.e., occurrence of developmental plateau and/or regression of acquired cognitive and motor skills during the active phase of epilepsy due to abundant epileptiform activity), whereas other syndromes, such as epilepsy with myoclonic absences and EEM, may be associated with a developmental and epileptic encephalopathy (i.e., pre-existing developmental delay plus subsequent regression of acquired skills or developmental plateau due to abundant epileptiform activity), an epileptic encephalopathy (as above mentioned), or a developmental encephalopathy (i.e., developmental impairment observed prior to epilepsy onset and no association could be observed between frequent epileptic activity and regression/further slowing) (Hirsch et al., 2022; Fisher et al., 2017).

However, these GGE syndromes have also been characterized by some overlap with classical IGE syndromes, as illustrated by higher rates of IGE syndromes in relatives of individuals with EEM, epilepsy with myoclonic absences and myoclonic epilepsy in

infancy (Sadleir et al., 2012; Hirsch et al., 2022). From a clinical point of view, these GGE syndromes have been associated with a reduced rate of seizure control and with higher prevalence of intellectual disability compared with classically IGE syndromes.

### 2.2.1 *Myoclonic epilepsy in infancy*

Myoclonic epilepsy of infancy is a rare self-limited epilepsy syndrome characterized by brief myoclonic seizures in previously healthy and developmentally normal children with onset in the first three years of life (Dravet and Bureau, 1981). Two-third of cases occurs spontaneously, and in one-third, seizures are triggered by a sudden noise, touch, or light (reflex variant) (Ricci et al., 1995). Myoclonic epilepsy of infancy is an uncommon condition and accounts for 1% to 2% of infantile/childhood epilepsy, and males are twice as likely to be affected than females (Caraballo et al., 1997). Although a family history of febrile seizures and/or epilepsy could be seen in one third of patients, single-gene variants have been sporadically reported and a complex pattern of inheritance has been hypothesized also in this syndrome (Campostrini et al., 2018).

Seizure onset occurs mostly in the age group of 6 months to 2 years, even though it can happen as young as four months and up to five years of age (Dravet et al., 1992). Myoclonic seizures are the prominent seizure type and are mandatory for the diagnosis. They are characterized by jerks involving predominantly the head and upper extremities (Lin et al., 1998). A personal history of febrile seizures has been described approximately in one-third of cases (Dravet et al., 1992). Response to ASMs is usually excellent, with almost 80% of patients being responsive. After 3 to 5 years from onset, ASM therapy could be gradually weaned over months. The reflex variant with acoustic and somatosensory triggered myoclonus may not need treatment or ASM could be used and eventually weaned after one year, whereas the reflex variant with photosensitivity is

usually more challenging to control (Wheless et al., 2007). Although seizures typically are self-limited and resolve during childhood, some patients may have a recurrence during adolescence. Afebrile generalized tonic-clonic seizures can occur in 20% of patients, and evolution towards JME has been rarely reported (Dominguez-Carral et al., 2014).

*2.2.2. Epilepsy with myoclonic absences*

Epilepsy with myoclonic absences is a rare epilepsy syndrome whose definition has been mainly made on the basis of the characteristic seizure type (Genton and Bureau, 2006). Epilepsy with myoclonic absences has an incidence of <1% and is more common in males, and the onset of epilepsy has been usually described at 7 years. Epilepsy with myoclonic absences has been reported to result from inborn or acquired brain damage (Bureau & Tassinari, 2005), chromosomal abnormalities (Guerrini et al., 1990; Elia et al., 1998), polygenic pattern of inheritance, and only sporadically, from single-gene variants (e.g. SYNGAP1, GLUD1, SETD1B) (Bahi et al., 2008; Klitten et al., 2011; Hiraide et al., 2019). Approximately 20% of patients have been described to display a family history of generalized epilepsy, supporting a strong genetic component in the context of this etiological heterogeneity.

Myoclonic absences are described as typical absences with sudden onset and offset that are associated with axial and arms hypertonia (the subject usually bends forward and slightly raises their shoulders and arms), and jerks synchronous with the SW discharges (Iyer et al., 2017; Zanzmera et al., 2016; Carter et al., 2022). Seizures usually occur many times a day, and awareness of the jerks may be maintained. Seizures could be challenging to control, and up to 45% of patients may have developmental delay or learning disorders (Bureau and Tassinari, 2005; Genton and Bureau, 2006). Tonic-clonic seizures have been

described and have been associated with a worse neuropsychiatric outcome (Carter et al., 2022). Refractory patients may also evolve towards epileptic and developmental encephalopathies, including the Lennox-Gastaut syndrome and others (Panayotopoulos, 2008; Genton and Bureau, 2006).

*2.2.3 Epilepsy with myoclonic-atonic seizure*s

Epilepsy with myoclonic-atonic seizures is an uncommon childhood epilepsy syndrome that accounts for 1 to 2% of all childhood-onset epilepsies (Kelley and Kossof, 2010). It has been described to be more common in men (male to female ratio, 2:1), and the onset of seizures could occur between 6 months and 6 years of age, with a peak between 2 and 4 years (Tang and Pal, 2012).

A genetic component with multifactorial inheritance has been commonly reported, with a positive family history of seizures of variable types in approximately 1/3 of cases (Trivisano et al., 2011). However, a wider range of single-gene variants has been found compared with other GGE syndromes, including SCN1A, SCN1B, GABRG2, SCL2A1, SLC6A1, STX1B and SYNGAP1 (Wolff et al., 2017; Schubert et al., 2014; Carvill et al., 2015, Specchio et al., 2022).

Myoclonic-atonic seizures have been characterized by brief myoclonic jerk affecting the proximal muscles and followed by a very brief atonic component, which may determine a head nod or a more prominent abrupt fall. This type of seizure has been considered as mandatory for the diagnosis in recently published diagnostic criteria (Specchio et al., 2022). Other seizures that have been frequently described include myoclonic seizures, absences, and generalized tonic–clonic seizures (which often represent the presenting seizure type) (Oguni et al., 2005; Kilaru et al., 2007). Approximately one quarter of children have a history of a febrile seizure (Neubauer et al., 2005), and family studies

highlighted a high prevalence of siblings with genetic epilepsy with febrile seizure plus phenotypes (Singh et al., 1999).

Prior to epilepsy onset, almost two third of cases present with normal development (Joshi et al., 2021). The onset of epilepsy is often abrupt, with explosive “stormy” onset of many seizures. Seizures often are drug-resistant, particularly during the high seizure frequency (explosive or stormy) phase, and recurrent bouts of nonconvulsive status epilepticus with increased frequency of other generalized seizure types are seen (Oguni et al., 2002).

During this phase, ataxia, developmental plateauing or regression, predominantly on behavior and executive functions, are often evident. Despite seizures being drug-resistant initially, two thirds of children could achieve remission, usually within 3 years of onset, and can be weaned off antiseizure therapies (Specchio et al., 2022). In the remaining third, persisting seizures, cognitive impairment, and behavioral abnormalities are often seen. Once seizures are controlled and the EEG improves, developmental progress is usually seen (Caraballo et al., 2013; Eschbach et al., 2018).

*2.2.4 Epilepsy with eyelid myoclonia*

EEM was originally described in 1977 by Peter M Jeavons, by the occurrence of marked photosensitivity (PS), eye closure sensitivity (ECS), and eyelid myoclonia (EM) with or without absences (Jeavons et al., 1977). Due to clinical overlap with other epilepsy syndromes, including IGE, focal genetic photosensitive epilepsies, structural epilepsies, and genetic epileptic encephalopathies, many authors had not considered EEM as a unique nosological entity until recently (Ferrie et al., 1996, Striano et al., 2009). However, in view of growing evidence pointing on specific electroclinical, genetic and neurophysiological features, EEM has been recently listed as a definite epilepsy syndrome (Specchio et al., 2022).

In this setting, a functional magnetic resonance (fMRI) study performed in EEM revealed that eye closure selectively determined an increased blood oxygen–level dependent (BOLD) signal over visual cortex, posterior thalamus, and eye movement control areas, suggesting a focal hyperexcitability over the visual system in the context of a generalized cortical hyperexcitability (Vaudano et al., 2014). Conversely, several functional connectivity studies highlighted that other IGE syndromes are characterized by more prominent network changes over prefrontal, frontocentral and frontal cortices (Elshahabi et al., 2015; Vorderwülbecke et al., 2022). Moreover, family studies and twin studies also supported a distinct genetic background of EEM compared with other GGE syndromes (Adachi et al., 2005; Sadleir et al., 2012). In particular, Sadleir and collaborators analysed 18 individuals with EEM from 15 different families and accurately phenotyped all available family members (Sadleir et al., 2012). They found that EEM probands were more commonly characterized by a family history of genetic epilepsy with febrile seizures plus (GEFS+) compared with other analysed IGE syndromes, suggesting a more relevant role of GEFS+ susceptibility genes in EEM and further supporting its recognition as a distinct epilepsy syndrome.

When considering the clinical features of EEM, onset of epilepsy has been mainly described during childhood, with a peak around seven years of age (Smith et al., 2018). A striking female preponderance has been consistently reported, with a female to male ratio ranging from 2:1 to 4:1 (Covanis, 2005; Zawar et al., 2022a). Prevalence has been variably reported from 7.3% to 13% among GGE syndromes, perhaps as a result of underreporting, under recognition and/or under diagnosis (Covanis et al., 2005). A family history of epilepsy has been described in up to one third of patients, and a complex inheritance has been hypothesized in the majority of cases. However, few genetic variants

(mainly in SYNGAP1, KIA02022/NEXMIF, RORB and CHD2 genes) have been identified in a small number of patients (Zawar and Knight, 2021; Mayo et al., 2021).

The onset of seizures in EEM has been characterized by the occurrence of frequent daily episodes of EM associated with an upward deviation of the eyes and sometimes with head retropulsion/extension. These seizures have been generally described as brief (1-5 seconds) and typically triggered by eye closure and could be associated or not with mild impairment of awareness (Panayiotopoulos, 2010). GTCS, spontaneous or light-induced, have been usually described to occur during the long-term follow-up, although they may represent the seizure type at onset in a minority of cases (Panayiotopoulos et al., 1996). A considerable quote of patients may experience self-induced seizures, typically induced by light stimuli and often associated with an handwaving behaviour mimicking an intermittent light stimulation (Darby et al., 1980; Fisher et al., 2022). Febrile seizures (FS) have been reported to occur in relatively high number of patients. Up to 20% of patients could develop EM status epilepticus, with repetitive, recurrent EM associated with mildly impaired awareness and responsiveness (Caraballo et al., 2009). Cognitive abnormalities, including intellectual disability (ID) and borderline intellectual functioning have been described in some patients, and usually range from mild to moderate (Parisi et al, 2011; Zawar and Knight, 2021). Psychiatric comorbidities have been reported as relatively common, with recent reports suggesting a high prevalence of behavioural abnormalities, including ADHD (Nilo et al., 2021; Morea et al., 2021).

In a quote of patients, sporadic myoclonic jerks over districts other than eyelids could also be observed during the disease course, although some controversies exist regarding the classification of EEM patients showing this seizure type (Parisi et al., 2011). Indeed, previous case reports showed marked clinical overlap between EEM and JME (Destina Yalcin et al., 2006), with patients evolving from one condition to the other, leading

several authors to consider myoclonia in body districts other than eyelid as an exclusion criterion for EEM (Striano et al., 2002).

The interictal electroencephalography (EEG) of EEM has been characterized by a normal background activity, and by the occurrence of SWD, PWD and polyspikes, predominantly occurring over the posterior regions (Zawar et al., 2022b). Non-rapid eye movement (NREM) sleep and awakening have been reported to activate these discharges, as in other GGE syndromes (Nilo et al., 2021). PS has been described as almost invariably present, although its clinical and EEG expression may decrease with age and could be modified by ASMs (Fischer et al., 2022). ECS has been reported as the other distinctive EEG trait and should be distinguished by fixation off sensitivity (FOS) by the brief duration of discharges, typically occurring within 1-3 seconds after eye closure and terminating after few seconds (Sevgi et al., 2007; Yalcin et al., 2021). Focal EEG abnormalities have been reported also as common, and often localized over the occipital areas, driving some authors to hypothesize a focal occipital origin of this epilepsy syndrome (Viravan et al., 2011; Niu et al., 2022).

The differential diagnosis of EEM should comprise IGE syndromes with absence seizures (namely CAE, JAE and JME), photosensitive occipital epilepsy (characterized by focal visual seizures which are not encountered in EEM), and other early-onset epilepsies with myoclonus and photosensitivity, including rare monogenic epilepsies (Smith et al., 1996; Elmali et al., 2020; Vlaskamp et al., 2019; Canafoglia et al., 2015; Sadleir et al. 2020). Furthermore, clinicians should be aware that EM clinically overlap with non-epileptic movement disorders, such as facial tics and compulsive blinking, and epilepsy may go unrecognized until generalized tonic-clonic seizures (GTCS) manifest (Kent et al., 1998, Reyhani et al., 2020).

VPA and levetiracetam (LEV) represent the most common ASMs used in EEM, due to their effectiveness on the seizure types occurring in EEM (Parissis et al., 2014; Di Bonaventura C et al., 2005). However, VPA may be undesirable in female EEM patients with childbearing age, due to its pronounced teratogenicity and other adverse effects (e.g., weight gain, endocrinological side effects, other cosmetic side effects, etc.) (Christensen J et al., 2021; Isojärvi et al., 1996), and in these cases, LEV has been considered a useful substitute (Striano et al., 2008). Ethosuximide, lamotrigine, clobazam, clonazepam, zonisamide, brivaracetam, topiramate, primidone and phenobarbital could also be used, due to their effectiveness on different generalized seizure types (Cerulli Irelli et al., 2021; Beydoun et al., 2012; Stephen et al., 2020). Case studies documented worsening of seizures with oxcarbazepine and cannabidiol (Menon et al., 2011; Zawar et al., 2021), whereas ketogenic diet has been found to be helpful in a few patients with EEM (Reyhani et al., 2020; Madaan et al., 2019). ASMs often ameliorate the seizures associated with EEM, but usually do not provide full control, with up to two-third of patients reported to be drug resistant in previous studies (Giuliano et al., 2019; Caraballo et al., 2009; Covanis et al., 2015).

### *2.2.5 Assessment of current state of knowledge and research in epilepsy with eyelid myoclonia*

When focusing on previous literature, the majority of existing studies examining EEM have been conducted in small patient cohorts with relatively short follow-up, leading to uncertainties regarding the electroclinical features, the seizure outcome trajectories and the factors predicting long-term prognosis (Nar Senol et al., 2015). Moreover, a significant clinical heterogeneity has been described across EEM patients (Incorpora et al., 2002; Appleton et al., 1993; Giannakodimos et al., 1996): the age at onset may vary from early infancy to late adolescence, the presence and degree of intellectual disability

(ID) could differ across patients, as well as the combination of seizure types observed (Covanis et al., 2015; Scuderi et al., 2000). Capovilla and coworkers described a group of EEM patients showing ASM refractoriness, high rates of EM status epilepticus, and ID, highlighting for the first time the existence of a distinct and homogenous EEM sub-phenotype (Capovilla et al., 2009).

Despite this clinical heterogeneity, no study has investigated EEM with the use of modern clustering techniques. Cluster analysis is a statistical technique used to recognize natural patterns of subjects, i.e. to group them in such way that subjects in one group or cluster are more alike than subjects in different groups or clusters with regards to defined clinical characteristics (McLachan, 1992; Rodriguez et al., 2019). This statistical approach has been increasingly used in recent years because of the practical unmet clinical need to identify disease subtypes and stratify patients into distinct sub-phenotypes to improve health care delivery and research (Hendricks et al., 2021). In other medical diseases (e.g. asthma), cluster analysis helped identify specific patient characteristics related to disease and therapeutic response, allowing directed personalized medical intervention (Ortega et al., 2014)

Finally, in spite of the prominent female predominance observed in EEM, no study has investigated the sex differences in terms of both electroclinical features and prognostic factors. Previous studies conducted in other GGE syndromes (i.e., JME) highlighted pervasive differences in clinical presentation according to sex, with female patients displaying higher rate of absence seizures, photosensitivity and triggered seizures (Christensen et al., 2005; Kishk et al., 2019; Poleon et al., 2017; Wolf et al., 1986; Kasteleijn-Nolst Trenite et al., 2013a). These findings led previous authors to hypothesize sex differences in JME in terms of seizure susceptibility and cortical excitability (Puri et al., 2013; van Campen et al., 2016). The importance of a sex-stratified approach was

further supported by recent results highlighting the prognostic impact of catamenial seizures in different IGE syndromes (Choi et al., 2020; Cerulli Irelli et al. 2022a; Stevelink et al., 2022), suggesting the importance of cyclical changes of estrogen and progesterone in seizure control in generalized epilepsies (Verrotti et al., 2012). Moreover, no study has explored in EEM the possible impact of recent regulatory agencies' restrictions limiting the use of VPA in female patients, despite the prognostic relevance previously highlighted in other GGE syndromes (Tomson et al., 2015; Lawthom, 2018; Atalar et al., 2021).

## 3. Experimental section

In the experimental part of the thesis, we will illustrate the results from three original multicenter international studies conducted on the largest cohort of epilepsy with eyelid myoclonia (EEM) patients ever recruited. Indeed, during the past three years, we had the opportunity to coordinate an international study group including 20 epilepsy centers from 9 different countries (Italy, France, Argentina, Russia, Switzerland, United Kingdom, Turkey, Spain, Netherlands).

In the first study, we adopted strict criteria to define EEM, in view of the existing classification controversies, and we aimed to: 1) investigate seizure outcome and prognostic factors in a well-defined cohort of EEM patients during a long-term follow-up; 2) delineate, through a cluster analysis approach, the possible existence of disease sub-phenotypes corresponding to distinct prognostic trajectories.

In the second study, we decided to enlarge the cohort of EEM patients by including those with sporadic myoclonia in body district other than eyelids, to determine if they represent a distinct entity or could be properly classified as EEM. In addition, based on both the prognostic impact of age at epilepsy onset observed in the first work and the growing relevance of this clinical feature in defining homogenous subtypes in different neuropsychiatric disorders, we decided to perform an age-at-onset-based clustering of this enlarged cohort of EEM patients.

Finally, in the third study, due to the striking female preponderance documented in our cohort across all the disease sub-phenotypes identified, we investigated the possible existence of electroclinical and prognostic differences according to sex.

## 3.1 Study 1: Electroclinical Features and Long-term Seizure Outcome in Patients With Epilepsy with eyelid myoclonia

### Methods

#### Study participants, setting, and eligibility criteria

The study was conducted according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines as a retrospective multicenter cohort study. Data from patients followed from 1983 to 2020 at 16 different pediatric and adult specialized epilepsy outpatient clinics, most of them members of the EpiCARE European Reference Network for Rare and Complex Epilepsies, were retrospectively reviewed.

Patients were enrolled according to the following inclusion criteria: 1) history of eyelid myoclonia (EM) with or without absences; 2) history of photosensitivity (PS) and/or eye closure sensitivity (ECS); 3) EEG generalized spike-wave discharges (SWDs) and/or polyspike-wave discharges (PWDs); 4) absence of spontaneous or provoked myoclonia in body parts other than the eyelids; 5) normal neuroimaging (when available) and neurological examination; 6) follow-up for at least 5 years. We excluded patients with: 1) cognitive deficits other than borderline intellectual functioning and mild intellectual disability (ID) to minimize the risk of including patients with clear-cut epileptic/developmental encephalopathy (Striano et al., 2002); and 2) myoclonic jerks in body parts other than the eyelids to avoid including patients with juvenile myoclonic epilepsy (JME).

#### Clinical data collection and EEG assessment

Clinical charts were thoroughly reviewed for demographic data, family history of epilepsy, history of febrile seizures (FS), age at epilepsy onset, seizure types throughout the epilepsy course, occurrence of EM status epilepticus and self-induced seizures, drug

regimen changes, magnetic resonance imaging (MRI) findings (when available), psychiatric comorbidities, and follow-up duration. Follow-up information on seizure type(s), frequency, and treatment adherence was reviewed for each visit. The presence of borderline intellectual functioning and/or mild ID, as established by at least one standardized neuropsychological test, was noted for each patient.

Standard EEGs were reviewed to assess the following features: 1) background activity; 2) presence and characteristics of ECS and PS; 3) SWD and PWD occurrence and frequency; 4) presence of focal epileptiform abnormalities, defined as focal discharges confined to a single lobe; 5) asymmetry of SWDs or PWDs both in onset and amplitude; and 6) presence of focal slow waves. Sleep EEG recordings, if available, were reviewed to assess the presence of generalized paroxysmal fast activity (GPFA), defined as a generalized discharge of rhythmic polyspikes in beta frequency with a duration of at least 1 s (Sun et al., 2018).

**Clinical outcomes**

Different seizure outcome measures were assessed during follow-up in each patient. The primary endpoint was the occurrence of sustained terminal remission (STR), defined as a period of at least 4 consecutive years of freedom from all seizures at the last follow-up visit. The time from the first antiseizure medication (ASM) trial to STR was also obtained for each patient, corresponding to the time period from the first ASM trial to the last seizure before STR started. The occurrence of a 2-year remission from all seizure types during clinical history was also considered. Patients who did not achieve at least a 2-year remission during their history were considered to show a non-remission pattern. When at least a 2-year remission was achieved, two distinct patterns of seizure control, namely a relapse and remission pattern, were distinguished according to the occurrence or absence

of subsequent seizure relapses during follow-up. The time period to the first 2-year remission from the patient's history was also calculated for each patient to investigate the latency from the first ASM prescription to the initial medication response. Finally, the occurrence of a 2-year remission from generalized tonic-clonic seizures (GTCS) at the last follow-up visit was evaluated.

In addition, we noted the number of ASM trials during the disease course and the number of ASMs at the last follow-up visit. The recurrence of seizures after ASM withdrawal was also investigated, considering only patients with a follow-up of at least 12 months after ASM discontinuation.

**Cluster analysis**

The two-step cluster analysis (TSCA) approach was used to investigate the presence of distinct EEM endophenotypes and identify the electroclinical features characterizing these subphenotypes. TSCA is a hybrid cluster approach that performs group clusterization through a double-step procedure. It first separates groups using a distance measure and then chooses the optimal subgroup model through a probabilistic approach. This approach provides several advantages over more traditional clustering techniques since it permits the use of both categorical and continuous variables, the handling of outliers, and the selection of the number of clusters based on statistical measurements rather than arbitrary choice, and is highly reliable and reproducible (Benassi et al., 2020; Kent et al., 2014). The following variables were used to perform TSCA: 1) the presence of mild ID and/or borderline intellectual functioning; 2) a family history of epilepsy in 1$^{st}$ or 2$^{nd}$-degree relatives; 3) early-onset EEM (as defined below); 4) a history of GTCS; 5) a history of EM status epilepticus; and 6) prognostic patterns (i.e., remission, relapse, and non-remission), as defined above.

**Statistical analysis**

Each variable distribution was graphically analyzed in order to select the appropriate statistical tests and ensure the highest possible reliability of identified results. Among all variables, the distribution of the age at onset variable was tested and graphically analyzed, resulting in a non-normally distributed variable. Distribution analysis showed a multimodal pattern that was further analyzed through kernel density estimation (KDE) in order to identify underlying modes. Subsequently, Fisher-Jenks optimization algorithm was used to confirm KDE intervals and to identify the best cut-off for categorization of the variable, which was determined to be 8.5 years. Early-onset EEM was therefore defined as EEM with an age of seizure onset $\leq 8$ years (the main statistical analysis was also repeated using age at onset as a continuous variable, which yielded comparable results that are reported in Supplementary table 1). Categorical variables were compared through Fisher's exact test, while continuous variables were compared using Wilcoxon-Mann-Whitney test due to their non-normal distribution. Group tests were two-sided, with $p < 0.05$ considered statistically significant.

Kaplan-Meier estimates were performed in order to calculate the cumulative time-dependent probability of entering STR during follow-up. The time of entry into the analysis was the date of epilepsy diagnosis, and the time of the endpoint was the date of STR onset or the date of the last follow-up visit (depending on which occurred first), truncated at 40 years of follow-up. Cox proportional hazards model was used to investigate the association between STR occurrence and possible predictors based on previous studies. Results were presented as hazard ratios (HRs) with 95% confidence intervals (CIs).

Multivariable multinomial logistic regression analysis was used to assess the relation between prognostic patterns (dependent variables) and their possible clinical predictors using the remission pattern as a reference. Results were presented as odds ratios (ORs) with 95% CIs. Finally, a linear regression model was used to assess the relation between the number of ASMs at the last follow-up visit (dependent variable) and its possible clinical predictors.

## Results

### *General clinical features of the study cohort*

After identifying 301 potential EEM patients, 172 subjects (123 female, 71.5%) were included according to study criteria (the inclusion tree is represented in Supplementary Figure 1 in the supplementary material). The median age at epilepsy onset was 7 years (IQR 5-10) and the median follow-up duration was 14 years (IQR 8.3-23.8). A history of psychiatric comorbidities was found in 45 patients, among whom 18/45 (40%) were diagnosed with mood disorders, 23/45 (51.1%) with behavioral disorders, and 4 (8.9%) with psychotic disorders. Descriptive statistics of the cohort with main clinical and demographic data are summarized in Table 2.

### *Electroclinical characteristics*

EM with or without absences was the seizure type at epilepsy onset in 131 patients (76.2%), whereas GTCS was reported as the presenting seizure type in 41 (23.8%). All patients fulfilled the criteria of either PS or ECS. A history of either PS or ECS was found in 156 (90.7%) and 134 patients (77.9%), respectively, and a history of both PS and ECS was observed in 117 (68%) patients. During follow-up, 120 (69.8%) patients experienced GTCS at least once, and 22 (12.8%) had a history of EM status epilepticus. The occurrence of self-induced seizures during history was found in 17 patients (9.9%),

whereas a clear-cut catamenial worsening of EM and/or GTCS was reported in 15/123 (12.2%) female patients.

All but 6 patients showed spontaneous SWD/PWD during at least one standard EEG, whereas generalized discharges were only provoked by intermittent photic stimulation and/or eye closure in these 6 patients. SWDs were recorded in 144 (83.7%) subjects while PWDs were recorded in 131 (76.2%). SWD/PWD frequency was ≥ 4 Hz in 110 (64%) patients. Focal spike and/or sharp waves were reported in 36 subjects (20.9%), and asymmetric/asynchronous generalized discharges were found in 10 (5.8%). A total of 159/172 (92.4%) patients performed at least one sleep EEG during follow-up, and 8/159 (8.8%) were found to have generalized paroxysmal fast activity (GPFA) during sleep.

At the last year of follow-up, ECS persisted in 73/158 (46.2%) patients among whom this data was available, whereas PS was found in 81/161 (50.3%) patients.

*ASM treatment*

The most common first-line ASM was valproate (VPA) in 108/172 patients (62.8%), followed by levetiracetam (LEV) in 19 subjects (11%), ethosuximide (ESM) in 16 (9.3%), and lamotrigine (LTG) in 8 (4.6%). During follow-up, the median number of prescribed ASMs was 3 (IQR 2-4). At the last follow-up visit, all but 16 patients were on ASMs. The median number of ASMs used at the last follow-up was 1 (IQR 1-2, range 1-5) and 78/172 patients (45.3%) were on a polytherapy regimen (≥2 ASMs). The most used ASM at the last follow-up visit was VPA in 95/172 patients (55.2%), followed by LEV in 58 (33.7%) and LTG in 36 (20.9%). The most frequently used monotherapies at the last follow-up visit were VPA in 41 patients, LEV in 17, and LTG in 13, which were associated with the following 2-year remission rates, respectively: 68.3%, 77.8%, and 46.2%. Among those on a bitherapy regimen (63 patients), the most frequently observed

combination was VPA + LEV (13/63), which was associated with the highest 2-year remission rate (61.5% vs. 36%, p=0.1). ASMs used at the last follow-up visit with the respective 2-year remission rate are given in Supplementary Figure 2.

*Seizure outcome and prognostic factors*

During follow-up, 106/172 (61.6%) patients achieved at least a 2-year remission from all seizure types and the mean time from epilepsy onset to the first 2-year remission was 10.45 years (SD ± 10.89). Therefore, 66/172 (38.4%) subjects displayed a non-remission pattern, whereas 56 (32.6%) and 50 (29.1%) patients showed a remission and relapse pattern of seizure control, respectively. Multivariable multinomial logistic regression analysis showed that a longer follow-up duration (OR=1.04, 95% CI =1.01-1.08, p=0.02), a history of GTCS (OR=3.15, 95% CI =1.05-9.43, p=0.04), and a family history of epilepsy (OR=3.11, 95% CI=1.22-7.94, p=0.02) were associated with a relapse pattern of seizure control, whereas early epilepsy onset (OR=4.88, 95% CI=1.82-12.98, p=0.002), EM status epilepticus (OR=5.05, 95% CI=1.24-20.8, p=0.02), and a history of FS (OR=9.01, 95% CI=1.67-47.61, p=0.01) significantly predicted the non-remission pattern (see Table 3 for detailed multivariable multinomial logistic regression results).

STR was achieved in 68 (39.5%) patients, and mean time from epilepsy onset to STR was 14.05 years (SD ± 12.47). Early epilepsy onset (HR=0.41, 95% CI=0.24-0.70, p<0.001), a history of GTCS (HR=0.47, 95% CI=0.27-0.82, p=0.008), psychiatric comorbidities (HR 0.34, 95% CI=0.16-0.71), and a history of FS (HR=0.18, 95% CI=0.05-0.76) were significantly associated with a lower chance of entering STR according to the Cox proportional hazards model. Results of the Cox proportional hazards model are reported in Table 4 and the cumulative probability curves of significant prognostic factors are

illustrated in Fig. 1. At the last follow-up visit, 88/120 (73.3%) patients had achieved 2-year freedom from GTCS.

The persistence of ECS at the last medical observation was associated with significantly lower rates of 2-year remission at the last follow-up visit (25/73 vs. 53/85, p=0.001). In addition, the persistence of PS was also associated with lower rates of 2-year remission at the last medical observation (30/81 vs. 49/80, p=0.006).

ASM withdrawal was attempted in 62/172 (36%) patients: seizure freedom at least 1 year after ASM discontinuation was observed in 16/62 patients (25.8%). Among those who displayed seizure relapse after at least 1 year of ASM withdrawal (46/62, 74.2%), GTCS relapse was observed in 28 patients (28/35, 80%) with a previous history of GTCS. Patients with seizure relapse after ASM withdrawal had significantly higher rates of GTCS during their history (36/47 vs. 5/16, p=0.002), whereas no significant differences were found according to other variables.

When considering the number of ASMs at the last follow-up visit as a dependent variable, early epilepsy onset ($\beta = 0.20$, p=0.009) and history of GTCS ($\beta = 0.17$, p=0.02) were significantly associated with the use of higher numbers of ASMs at the last follow-up visit according to a multiple linear regression model (F=4.5, p<0.001). The results of multiple linear regression are reported in Supplementary Table 2 the Supplementary material section.

*Cluster analysis: identification of clinical EEM subtypes*

TSCA revealed two distinct clusters (86 patients per group) of EEM patients, with similar follow-up duration. The two clusters, hereinafter referred to as “EEM-only” (Cluster 1) and “EEM-plus” (Cluster 2) significantly differed in terms of age at epilepsy onset and cognitive abnormalities, with the latter showing a younger age at epilepsy onset and a

higher percentage of ID/borderline intellectual functioning (16.3% vs. 47.7%, p<0.001). In addition, EEM-plus patients were characterized by a higher proportion of FS (5.8% vs. 16.3%, p=0.049), self-induced seizures (4.6% vs. 15.1%, p=0.03), and EM status epilepticus (4.6% vs. 20.9%, p=0.002).

The two clusters had similar rates of mood disorders (EEM-only: 11.6% vs. EEM-plus: 10.5%, p=0.6) and psychotic disorders (1.2% vs. 3.5%, p=0.3), whereas EEM-plus patients had higher rates of behavioral disorders (8.1% vs. 17.4%, p=0.07) compared with EEM-only patients.

As far as EEG characteristics, EEM-plus patients were found to have higher rates of PS persistence at the last year of follow-up (37.2% vs. 57%, p=0.02), as well as higher rates of ECS (32.6% vs. 52.3%, p=0.01), compared with EEM-only patients. Additionally, GPFA during sleep was significantly more frequent among EEM-plus patients compared with EEM-only patients (3.5% vs. 12.8%, p=0.02), with a similar proportion of patients undergoing sleep EEG recordings during follow-up in the two clusters (95.3% vs. 89.5%, p=0.3).

When considering seizure outcome, EEM-plus patients showed a significantly higher rate of non-remission pattern (0 vs. 76.7%, p<0.001), a similar rate of relapse pattern (EEM-only: 31.4% vs. EEM-plus: 23.3%, p=0.3), and a significantly lower rate of remission pattern (68.6% vs 0, p<0.001) compared with EEM-only patients.

The two clusters did not significantly differ in terms of sex, family history of epilepsy in 1$^{st}$ or 2$^{nd}$ degree relatives, or GTCS history. The electroclinical differences between the two clusters are illustrated in Fig. 2, whereas all statistics and p values related to comparisons between clusters are reported in Table 5.

## Discussion

In this multicenter study, we evaluated the electroclinical characteristics and determined the prognostic factors for distinct epilepsy evolution patterns in a cohort of 172 EEM patients with a long-term follow-up. More than one third of our patients displayed a non-remission pattern, whereas remission and relapse patterns were found at almost equal rates in the remaining subjects.

Only 39.5% of our population achieved STR, with a median latency of 14.05 years. A similarly long delay was also observed when considering the interval from epilepsy onset to the initial medication response (time from onset to first 2-year remission = 10.45 years), suggesting a key role of age-related brain changes, as previously hypothesized for other photosensitive epilepsies (Jeavons et al., 1986; Panayiotopoulos et al., 2008; Regesta and Tanganelli, 1989). Among the investigated prognostic factors, early epilepsy onset was the most powerful predictor in our study and was significantly associated with both failure to reach STR and no-remission pattern of seizure control. Our observation is in line with previous findings in a much smaller subgroup (9 patients) published by Caraballo et al., who found treatment refractoriness in all patients with early-onset EEM (Caraballo et al., 2009). A previous history of FS was also significantly associated with both not converting to STR and with a non-remission pattern. The negative impact of FS on long-term seizure outcome was also recently highlighted in a GGE cohort and was attributed to genetic factors that may predispose patients to both FS and ASM refractoriness (Cerulli Irelli et al., 2020a; Mohanraj and Brodie, 2007). In accordance with this hypothesis, a family study conducted by Sadleir et al. revealed that generalized epilepsy with FS-plus was common among relatives of EEM patients, suggesting shared genetic determinants between these two syndromes (Sadleir et al., 2012). Furthermore, a

history of GTCS and psychiatric comorbidities significantly predicted failure to achieve STR, in line with previous observations across different epilepsy syndromes (Choi et al., 2020; Geithner et al., 2012; Gomez Ibanez et al., 2017). Finally, a previous history of EM status epilepticus was associated with a 5-fold increased risk of not experiencing remission throughout the course of EEM. This latter observation, together with the prognostic impact of an earlier age at epilepsy onset and a history of FS, may reflect shared underlying genetic components, as supported by the results of our cluster analysis.

In this study, we confirmed the existence of a subgroup of EEM patients with an insidious phenotype, referred to as "EEM-plus", and another more benign subgroup, referred to as "EEM-only". Subgroups of EEM patients characterized by a higher rate of moderate ID, status epilepticus, and ASM resistance have been previously described in small cohorts (Caraballo et al., 2009; Capovilla et al., 2009), but these observations have not yet been corroborated in larger cohorts with modern statistical approaches. The two EEM patient subgroups, as delineated here, differ to a great extent in terms of both their electroclinical features and long-term seizure outcomes. EEM-plus patients were younger at epilepsy onset and had higher rates of cognitive disturbances, EM status epilepticus, FS, GPFA and self-induction when compared with EEM-only patients. In addition, EEM-plus patients showed higher rates of poor response to ASMs, whereas EEM-only patients showed a favorable long-term seizure outcome, with two thirds of patients achieving a remission pattern of seizure control.

EEM has generally been recognized as an epilepsy syndrome with a high rate of ASM refractoriness regardless of the electroclinical characteristics of affected patients (Joshi et al., 2007; Giuliano et al., 2019). Based on cluster analysis, we have made a clear-cut distinction between EEM patient subtypes, with EEM-plus patients having a poor response to ASM and, thus, a less favorable long-term seizure outcome than EEM-only

patients. Further studies will clarify if the differences between EEM subtypes may be attributed to the underlying genetic substrate, with EEM-plus patients possibly harboring mutations in genes related to EEM and EEM-like phenotypes, such as *SYNGAP1*, *KIA02022*, and *CHD2*, which have been established as the most consistent genetic contributors in this setting (Mayo et al., 2021; Stamberger et al., 2021; Thomas et al., 2015; Vlaskamp et al., 2019).

In this study, for the first time we also explored ASM withdrawal in EEM patients. One third of EEM patients in our cohort discontinued ASMs during follow-up, in line with previous studies in JME patients (Syvertsen et al., 2019), and one fourth of these patients remained seizure-free after ASM discontinuation. A previous history of GTCS emerged as the only predictor of seizure recurrence in our study, suggesting caution when withdrawing ASMs in these patients. However, due to the retrospective nature of our study, we were unable to quantify GTCS prior to ASM withdrawal. This potential limitation prevented us from determining whether a single GTCS during history could have the same prognostic significance as multiple GTCS on seizure recurrence after ASM withdrawal.

In addition, we documented an EEM onset peak during mid-childhood, as well as a female preponderance (2.51:1) and high rates of family history of epilepsy, thus providing solid evidence in support of previous findings from much smaller cohorts (Caraballo et al., 2008; Giuliano et al., 2019; Nilo et al., 2021; Striano et al., 2002).

As far as treatment data, the association VPA + LEV was most frequently associated with 2-year remission at the last follow-up visit, and both these ASMs were also associated with the highest remission rates when used as monotherapy, in line with previous literature findings (Striano et al., 2008). VPA was also found to be the most frequently

prescribed ASM during the entire follow-up duration, followed by LEV. Based on our data, the decreased VPA use in female patients of childbearing age due to its well-known teratogenic adverse effects may eventually result in higher rates of seizure refractoriness in EEM patients (as observed in other GGEs) (Cerulli Irelli et al., 2020b), especially considering the striking female preponderance observed in this rare epilepsy syndrome.

The main limitations of our study are due to its retrospective design and the lack of systematic genetic testing in all patients, which may have contributed to the interpretation of our findings about prognostic factors and sub-phenotypes. However, the multicenter design, the large number of patients as compared to previous cohorts, the long-term follow-up, and the strict diagnostic criteria used to define EEM support the generalizability of our results. In addition, we adopted strict criteria to define EEM in order to avoid the inclusion of other myoclonic syndromes, such as JME, especially in later-onset patients (Striano et al., 2009). We thus chose to exclude patients with a history of myoclonic seizures involving body regions other than the eyelids, although their classification still represents a controversial topic. Similarly, while the exclusion of patients with moderate/severe ID allowed us to minimize the risk of including patients with clear-cut developmental/epileptic encephalopathy, their exclusion prevented us from definitively characterizing the entire spectrum of EEM sub-phenotypes with our cluster analysis, as previously suggested by Capovilla and collaborators (Capovilla et al., 2009).

In conclusion, our study reveals the clinical variables predicting the occurrence of sustained remission in EEM patients. In particular, early age at epilepsy onset appeared to be the most relevant predictor of poor seizure outcome. Moreover, using a large database with long-term follow-up data, we outlined the distinct prognostic patterns of this rare epilepsy syndrome. Finally, we identified two distinct EEM sub-phenotypes with strong implications in terms of seizure control and cognitive outcome.

### 3.2. Study 2: The spectrum of epilepsy with eyelid myoclonia: delineation of disease subtypes from a large multicenter study

**Methods:**

**Study participants**

Through the ongoing EEM study group, we collected the clinical data of 313 individuals followed from 1983 to 2020 recruited retrospectively from 20 sites across 9 countries. Institutional/regional ethics committees gave approval for this study and informed consent was obtained from all participants or their parents/caregivers.

Patients were enrolled according to the following criteria: 1) EM with or without absences; 2) history of PS and/or ECS; 3) EEG generalized spike-wave discharges (SWDs) and/or polyspike- wave discharges (PWDs); 4) normal neuroimaging (when available).

Patients with sporadic myoclonia in body regions other than the eyelids (hereinafter referred as body-MYO) were also included, as long as EM represented the predominant seizure type. Patients with predominant myoclonia in cranial regions other than eyelids were excluded, to avoid including patients with perioral/periorbital myoclonia with absences. Individuals with cognitive deficits other than borderline intellectual functioning and mild ID were excluded to avoid the enrollment of patients with a definite developmental/epileptic encephalopathy. Patients with a follow-up period (from the first antiseizure medication -ASM- prescription to the last visit) shorter than 24 months were also excluded, to allow a better prognostic characterization of the study participants. The clinical data of each patient were reviewed by ECI, CDB and PS to confirm the diagnosis of EEM according to the inclusion and exclusion criteria, as previously specified.

**Clinical and EEG assessment**

All medical charts and EEGs were reviewed to obtain demographic and electroclinical data, as described in the previous work of this thesis (Cerulli Irelli et al., 2022b). The occurrence of migraine with or without aura was also noted in each patient. The presence of borderline intellectual functioning and/or mild ID, as established by the Wechsler Intelligence Scale for children or adults, depending on the age at standardized investigation, was recorded for each patient. In addition, we created an extended pedigree for each participant reporting a family history of epilepsy, including the number of first- and second-degree relatives with epilepsy, and their specific epilepsy syndrome, based on patients' or relatives' interview, where applicable.

The presence of PS and/or ECS was defined as the occurrence of brief SWD/PWDs appearing within 1-3 seconds and lasting 1-4 seconds after eye closure. These should be clearly distinguishable from fixation-off sensitivity, defined as the occurrence of occipital or generalized epileptiform discharges induced by elimination of central vision and fixation. PS and/or ECS were mainly assessed by reviewing EEG recordings, provided that at least one EEG was available for each patient. However, to avoid an underreporting of ECS/PS in those patients who first presented to the outpatient clinic after remission of these epilepsy traits, we also took into account their occurrence based on clinical grounds alone.

For each patient the occurrence of 2-year remission from all seizure types during history, as well as the number and type of ASMs tried over time was evaluated. According to the definition by Kamitaki and colleagues, the failure of at least two adequately prescribed ASMs during history was regarded as ASM refractoriness, whereas patients with "rare breakthrough seizures due to missed doses of medication and occasional nondisabling

myoclonic seizures if these did not necessitate a change in management" were considered ASM-responsive (Kamitaki et al., 2022). The following ASMs were considered as adequately prescribed in the treatment of EEM: valproate, lamotrigine, ethosuximide, zonisamide, topiramate, levetiracetam, phenobarbital, primidone, clonazepam, clobazam and perampanel. Finally, seizure recurrence after ASM withdrawal was investigated in patients with ≥ 12-month follow-up after ASM discontinuation.

**Statistical analysis**

Data were presented as mean ± standard deviation (SD) or median with interquartile range (IQR) according to their normal or non-normal distribution, respectively. As regards AEO, the Kernel Density Estimation (KDE) was used to investigate its distributional pattern and assess the possible occurrence of multimodality (Silverman, 1981). Subsequently, the Fisher-Jenks algorithm was used to identify the optimal cut-offs to split the data and outline the underlying AEO-dependent clusters. Fisher-Jenks algorithm represents a class interval analysis that naturally integrates the KDE multimodal analysis. This algorithm improves the minimum distance analysis performed through K-Means, especially for unidimensional data (Khan, 2012). The identified AEO-related subgroups were compared by the Kruskal-Wallis or one-way ANOVA test in case of continuous variables and by the Fisher-Exact test in case of nominal variables. Finally, comparisons of the electroclinical characteristics between patients with or without body-MYO were performed by the Fisher Exact Test in case of nominal variables, whereas the Mann-Whitney U test and the unpaired-T test were used to compare continuous variables in case of their non-normal or normal distribution, respectively. Values of $p<0.05$ were considered statistically significant. Analyses were performed and figures were generated using R 3.5.1 (R Project for Statistical Computing, Vienna, Austria).

## Results

*Demographic Data*

Of the 313 EEM patients initially recruited, 267 were included according to the study methods. Reasons for exclusion were unconfirmed diagnosis of EEM in 35 cases and inadequate follow-up duration in 11.

The median AEO across the entire cohort was 7 years (IQR 5-10). When considering the specific seizure types, the median age at onset was 7 years (IQR 5-10) for EM, 12 years (IQR 10-15) for GTCS, and 14 years (IQR 8-17) for body-MYO (Figure 3).

Kernel density estimation revealed a trimodal distribution of AEO across the entire cohort (Figure 2), and Fisher-Jenks algorithm defined 6.5 years and 10.5 years as the best cut-offs for splitting the data into three AEO-dependent subgroups (Figure 4), namely: early-onset EEM (EO-EEM), including 118 patients (44.2%) with a mean AEO of 4.3 years (standard deviation -SD-) ± 1.54, intermediate-onset EEM (IO-EEM), including 87 patients (32.6%) with AEO of 8.5 years (SD ± 1.07), and late-onset EEM (LO-EEM), including 62 patients (23.2%) with AEO of 13.1 years (SD ± 1.76).

*Clinical characteristics*

The AEO subgroups did not differ in terms of sex distribution, follow-up duration, personal history of febrile seizures, self-induced seizures and EM status epilepticus. EO-EEM showed a higher rate of mild ID ($p$=0.002) and psychiatric comorbidities ($p$=0.009), whereas IO-EEM had the highest rates of family history of epilepsy in 1$^{st}$- and 2$^{nd}$-degree relatives (p=0.01). Finally, LO-EEM was associated with a higher rate of GTCS ($p$=0.006) and more frequently experienced body-MYO ($p$=0.03). A family history of EEM was more frequent in IO-EEM and LO-EEM compared with EO-EEM ($p$=0.02). As to EEG findings, the only significant difference between the groups lay in the proportion

with persistent PS at the last follow-up, which was higher in EO-EEM ($p$=0.04). The detailed clinical characteristics of the three AEO subgroups are illustrated in Table 6 (Table 6).

When focusing on body-MYO, we found that 58 individuals (21.7%) experienced them at some point during the disease course, but in only one case were they the presenting seizure type. In patients with body-MYO (from now on referred to as 'body-MYO+' patients), the age at onset of both EM and GTCS was significantly higher compared with the other study participants (Figure 5). In addition, a family history of both EEM (8.6% vs 4.8%, $p$=0.3) and JME (5.2% vs 1.9%, $p$=0.2) was slightly more common in body-MYO+ patients, whereas the proportion of participants with epilepsy in 1$^{st}$- and 2$^{nd}$-degree relatives did not vary with the presence of body-MYO.

Body-MYO+ patients were more likely to develop GTCS during follow-up ($p$=0.002) and report migraine with/without aura compared with the other study participants ($p$<0.001). Other clinical characteristics, including history of borderline intellectual functioning or mild ID, febrile seizures, psychiatric comorbidities, EM status epilepticus and self-induced seizures did not differ according to the presence of body-MYO (Table 7).

Finally, a similar proportion of patients with and without body-MYO had ECS and PS both at disease onset and at the last follow-up, and the rate of focal EEG findings was also comparable between these two subgroups (Table 7). Conversely, bursts of PWDs were recorded in a lower proportion of body-MYO+ patients compared with the remaining cohort (59.3% vs 73.9%, $p$=0.036).

*ASM treatment and seizure outcome*

The three AEO-subgroups did not differ in terms of ASMs used at first and last medical observation, except for lamotrigine, which was significantly more frequently used as first-

line monotherapy in LO-EEM (Supplementary figure 3). ASM withdrawal was more frequently attempted in IO-EEM compared with the two other subgroups (EO-EEM 33.1% vs IO-EEM 44.8% vs LO-EEM 25.8%, *p*=0.046), whereas seizure recurrence after withdrawal did not differ significantly between AEO-subgroups (EO-EEM 73.7% vs IO-EEM 74.4% vs LO-EEM 73.3%, *p*=1).

ASM refractoriness was found to be significantly more frequent in EO-EEM compared with IO-EEM and LO-EEM [EO-EEM: 75/118 (63.6%) vs IO-EEM: 41/87 (47.1%) vs LO-EEM: 31/62 (50%), *p*=0.04], and a trend towards statistical significance was also observed for higher rates of polytherapy regimen (≥ 2 ASMs) at the last follow-up visit in the same subgroup [EO-EEM: 60/118 (50.8%) vs IO-EEM: 30/87 (34.5%) vs LO-EEM: 27/62 (43.5%), *p*=0.06]. Two-year remission during history appeared slightly more common – though not significantly - among individuals who were older at epilepsy onset [EO-EEM: 68/118 (57.6%) vs IO-EEM: 55/87 (63.2%) vs LO-EEM: 35/62 (72.6%), *p*=0.1].

When focusing on body-MYO, the only significant difference in ASM trials lay in the use of ethosuximide at the last follow-up visit, which was less common among body-MYO+ patients compared with the rest of the cohort (1.9% vs 16%, p=0.005). ASM refractoriness, 2-year remission during history and recurrence after ASM withdrawal did not differ according to the presence of body-MYO during follow-up (see Table 7).

*Genetic data*

A total of 24/267 (9%) patients in the study cohort underwent whole exome sequencing. Among them, 6 patients harboured pathogenic variants in CHD2, two patients in NEXMIF and one in SCN8A.

## Discussion

*Clinical characteristics and family history of epilepsy according to AEO*

In this study, we highlighted the existence of remarkable electro-clinical differences among EEM patients according to AEO. Through statistical modeling on the largest cohort of EEM patients so far reported, we demonstrated that AEO displays a trimodal distribution, thus revealing three different EEM subtypes. Indeed, in several medical conditions age at onset has been previously identified as an important factor in defining homogenous disease clusters, with crucial genetic, clinical and prognostic implications (Bellivier et al., 2001; Marini et al., 2003; Nicolson et al., 2004).

The largest group identified was EO-EEM, which was characterized by the highest rates of ID, psychiatric comorbidities and ASM refractoriness. Further than confirming previous findings as to the negative impact of early age at onset in this epilepsy syndrome, both in terms of neuropsychiatric profile and seizure outcome (Caraballo et al., 2009; Cerulli Irelli et al., 2022b), we identified for the first time a significant correlation between AEO and family history of epilepsy. Indeed, EO-EEM patients showed the lowest rate of family history of epilepsy compared with the other subgroups, suggesting a likely more prominent role of *de novo* mutations in this EEM subtype, as hypothesized for other epilepsies and neurodevelopmental disorders (Epi4K consortium, 2013; Hamdan et al., 2014). Conversely, the higher frequency of positive family history of EEM found in both IO-EEM and LO-EEM suggests a stronger influence of inherited genetic burden in these two subtypes.

LO-EEM was the smallest group, including patients with epilepsy onset during adolescence. Adolescent-onset EEM had the highest rates of body-MYO and GTCS over the course of the disease, suggesting that these patients may lay at the farthest end of the

EEM spectrum, at the border of IGE, as hypothesized in the latest classification framework proposed by ILAE (Hirsch et al., 2022). Finally, IO-EEM could be considered in all respects as the "pure" EEM sub-phenotype, characterized by electro-clinical findings consistent with the original description by Jeavons (Jeavons et al., 1977).

A striking female preponderance, as well as high rates of PS, ECS, febrile seizures, EM status epilepticus and self-induced seizures, were found in all AEO-dependent subgroups, thus emerging as consistent hallmarks along the entire EEM continuum (Covanis et al., 2015).

*Is EEM with sporadic myoclonia in other body regions a distinct clinical entity?*

EEM associated with sporadic body-MYO has been classically considered as an intermediate phenotype between EEM and JME. In the present study we provided an extensive electro-clinical characterization of patients with body-MYO, revealing striking electroclinical differences between them and previously reported JME cohorts (Baykan et al., 2009; Cerulli Irelli et al., 2022a; Kasteleijn-Nolst Trenité et al., 2013b). First, febrile seizures appeared more frequent in our body-MYO+ patients (as well as in the whole study population) compared with well-defined cohorts of JME and other IGEs, reinforcing the hypothesis of a shared genetic background between EEM and generalized epilepsies with febrile seizures plus (Sadleir et al., 2012). Second, body-MYO+ patients showed strikingly higher rates of PS, ECS, borderline intellectual functioning and ID compared with JME, as well as higher rates of EM status epilepticus and self-induced seizures (Baykan et al., 2009; Cerulli Irelli et al., 2022a; Kasteleijn-Nolst Trenité et al., 2013b).

Conversely, we did not observe remarkable familial, electroclinical and prognostic differences between body-MYO+ and body-MYO- participants. Overall, our data suggest

that body-MYO+ patients should be set apart from JME since they suit well the complex continuum of EEM.

Nevertheless, a few phenotypic traits beyond the AEO mentioned above differed between body-MYO+ patients and the rest of our cohort. In particular, the significantly lower rate of PWDs, along with the higher proportion of patients showing GTCS in the body-MYO+ subgroup, suggests a peculiar pathophysiological background in these patients. In line with this hypothesis, we also found a significant association between migraine with/without aura and a history of body-MYO, as recently observed in a large cohort of idiopathic/genetic epilepsies as well (Atalar et al., 2022).

*EEM as a spectrum disorder*

In the previous work of this thesis (Cerulli Irelli et al., 2022b), we outlined two distinct EEM sub-phenotypes which differed to a great extent in terms of electroclinical features and long-term outcome: namely, the "EEM-plus" subgroup, with lower AEO, high rates of ID and ASM refractoriness, and the "EEM-only" subgroup, showing a more favorable prognostic profile. In the present study, after expanding the initial cohort by including patients with body-MYO, we confirmed the existence of remarkably different AEO-dependent sub-phenotypes. Interestingly, the EO-EEM cluster greatly overlaps with the previously described "EEM-plus" subgroup, with respect to its neuropsychiatric profile and seizure outcome, and shares clinical features with developmental and epileptic encephalopathies (DEEs). Conversely, IO-EEM is akin to the above-mentioned "EEM-only", considering its "pure" phenotype and the favorable response to ASMs. In addition, in this study we could identify a third subgroup, i.e., LO-EEM, more closely resembling the clinical and family features of JME, in spite of its distinct traits.

Overall, our data suggest that EEM should be considered as a spectrum disorder, encompassing a wide range of disease subtypes characterized by a variable combination of different ages at epilepsy onset, family history of epilepsy, seizure types, response to treatment, neuropsychiatric profiles, and neurological comorbidities. Our findings showed, once again, the thin line - and overlapping borders – existing between and within different clinical entities in the context of generalized epilepsies (Berkovic et al., 1987; Cerulli Irelli et al., 2022c; Johannesen et al., 2016).

The main limitation of this study arises from the lack of systematic genetic testing, which could have helped us interpret our findings, especially regarding the identified EEM subtypes. In addition, our retrospective study design entails several potential confounders, especially recall and inclusion biases, with the potential enrollment of some patients with EEM look-alike syndromes (e.g., perioral myoclonia with absences, Sunflower syndrome, JME and childhood absence epilepsy evolving to JME). In addition, the long follow-up of the study along with its retrospective nature prevented us from collecting more detailed information regarding seizure recurrence after ASM withdrawal (e.g., number and type of ASMs, age, and duration of seizure freedom at the time of withdrawal, etc.), which may have helped us in the interpretation of data about seizure recurrence. Furthermore, we decided to exclude patients with moderate/severe ID, to avoid including patients with clear-cut DE/DEE, who could have biased our analyses regarding the age at epilepsy onset; however, the exclusion of these patients may have prevented us from defining the entire spectrum of EEM subphenotypes. Finally, the epilepsy syndrome of the participants' relatives was identified mainly through patients' interviews, possibly determining some classification errors. Conversely, the large sample size and the multicenter design represent the main strengths of our study.

In conclusion, through an innovative statistical approach, we identified homogenous EEM subtypes according to AEO, characterized by distinct electroclinical and familial features. Our observations shed new light on the spectrum of clinical features of this generalized epilepsy syndrome and may help clinicians towards a more accurate classification and prognostic profiling of EEM patients.

### 3.3. Study 3: Sex-based electroclinical differences and prognostic factors in epilepsy with eyelid myoclonia

**Methods:**

*Study participants and data collection*

The clinical data of 313 individuals followed from 1983 to 2020 recruited from 20 sites across 9 countries were retrospectively reviewed, as specified in the previous work (Cerulli Irelli et al., 2022d).

Information about inclusion and exclusion criteria have been thoroughly described in the second study of this thesis (Cerulli Irelli et al., 2022d), as well as the methods for clinical and EEG assessment.

*Outcome assessment*

The primary outcome for this study was the occurrence of drug resistance at the last follow-up visit, as defined by the international league against epilepsy (ILAE) (Kwan et al., 2010). The recurrence of epileptic seizures as a probable consequence of poor adherence to treatment and/or attempts at AED tapering/withdrawal was considered as "pseudo-resistance" and did not contribute to the outcome definition.

**Statistical analysis**

Descriptive statistic methods and data visualization were used to assess data distribution. Comparisons of demographic and electroclinical variables between sexes were performed with independent sample t-test and the Mann-Whitney U test in cases of normal and non-normal distribution, respectively. Comparisons across proportions were performed with Fisher's exact test. To investigate the sex-specific factors associated with drug resistance, a dedicated multivariable logistic regression model was developed separately in each sex

(M1 model for men, M2 model for women). All variables showing a p value < 0.2 at univariable analysis were included in the multivariable model, whereas follow-up duration was retained in both models as potential confounder.

**Results**

*Demographic and electroclinical differences according to sex*

A total of 267 patients (195 women) were included in the study. Female and male individuals did not differ in terms of age at epilepsy onset, follow-up duration, and age at the last follow-up visit. Female patients were found to have a significant higher rate of migraine during history, combined with higher ECS and PS persistence at the last visit, whereas male patients showed a significant higher rate of intellectual disability/borderline intellectual functioning.

The two groups did not differ in terms of the number of ASMs used during follow-up, whereas valproate use at the last visit was significantly more frequent in men compared to women. See table 8 for a detailed comparison of demographic and clinical characteristics between females and males, as well as for the ASM used in both sexes (Table 8).

*Sex-specific determinants of drug resistance*

Drug resistance at the last follow-up visit did not significantly differ according to sex, although was found to be slightly higher in females [88/267 (45.1%) vs 27/72 (37.5%), p=0.3).

M1 revealed that age at epilepsy onset, catamenial seizures, psychiatric comorbidities and eyelid myoclonia status epilepticus were significantly associated with drug-resistance, whereas a borderline statistical significance was found for self-induced seizures (area under the curve-AUC- of the multivariable model = 0.71). When considering male

patients, M2 showed that a personal history of febrile seizures (FS) was the only factor associated with drug resistance (AUC = 0.62). See Table 9 for odds ratios and confidence intervals of both multivariable models (Table 9).

**Discussion**

In this study we highlighted the existence of electroclinical differences according to sex in a large cohort of patients with EEM, along with sex-specific determinants of drug resistance.

When focusing on electroclinical differences, female patients displayed a higher rate of PS and ECS persistence at the last visit, possibly reflecting the impact of sex factors on degrees of excitability of brain networks underlying these EEG patterns (Vaudano et al., 2014; Siniatchkin et al., 2007). Occipital cortex hyperexcitability has been hypothesized as a relevant pathophysiological mechanism in both EEM and ECS/PS, and hormonal factors along with genetic and epigenetic mechanisms may determine a higher occipital hyperexcitability in females, also possibly accounting for the striking female preponderance observed in this epilepsy syndrome. In accordance with this hypothesis, female patients displayed a higher rate of migraine with/without aura, in which the primary role of occipital cortex hyperexcitability has been repeatedly demonstrated (Gunaydin et al., 2006). Accordingly, in drug-naïve JME patients, a higher motor cortex excitability was found by means of transcranial magnetic stimulation in females patients compared with men (Puri et al., 2013).

Furthermore, we also found a higher rate of cognitive abnormalities (considering together intellectual disability and borderline intellectual functioning) in male patients compared with the female counterpart, which prompt future genetic-based studies to evaluate the possible impact of X-linked variants (Stamberger et al., 2021). When considering ASM

treatment, we also documented systematic differences in ASM exposure between females and males, showing a significant higher use of LEV in the formers and a significant higher use of VPA in the latters, as previously observed in different GGE syndromes (Virta et al., 2018).

In the present study, prognostic factors previously identified in EEM (namely eyelid myoclonia status epilepticus, age at epilepsy onset and psychiatric comorbidities), could be confirmed in females only (Cerulli Irelli et al., 2022b; Caraballo et al., 2009). Conversely, FS, a strong predictor of a reduced chance of sustained remission in a previous study by our group, was found to be significantly associated with the outcome only in the male sex. Based on this observation, we might hypothesize that physiological sex differences could confer distinct protection against early-life neurological insults, such as FS, as suggested in preclinical studies (Kloc et al., 2022).

Moreover, we were able to highlight for the first time the impact of catamenial seizures on seizure outcome in EEM. Catamenial seizures have been increasingly identified as an important prognostic factor in different GGE syndromes in previous well-designed studies, but they have never been investigated in EEM (Cerulli Irelli et al., 2022a; Choi et al., 2020; Shakeshaft et al., 2022; Stevelink et al., 2022). The cyclical changes in estrogens and progesterone circulating levels are widely accepted to play a crucial role in the development of catamenial seizures (Hattemer et al., 2007), through a modulation of cortical excitability or changes in ASM plasmatic dose due to pharmacokinetic interactions (Shavit et al., 1984).

Lastly, when comparing the discriminative ability of the logistic regression models, we found a better AUC in females compared to men, suggesting that other unmeasured variables could play a role in the development of drug resistance in male patients.

The retrospective design of our study represents the most important limitation in the interpretation of our findings, whereas the large sample size and the multicenter design with 18 involved epilepsy centers may support their generalizability. Moreover, the lower use of VPA observed in females may account for some of the electroclinical differences observed between sexes. For instance, the significant higher rate of PS and migraine in these patients could also be partially explained by the differential use in VPA, considering the well-known role of this ASM in the treatment of these conditions (Cerulli Irelli et al., 2020b; Gaudio et al., 2022).

In conclusion, our study confirms the relevance of a sex-personalized approach in epilepsy care and research and opens the way to future studies exploring the underlying pathophysiological and biological significance of the observed sex-based differences. A clinical and research approach based on sex could be of paramount importance in context of GGE, considering the prognostic relevance of sex-based factors (e.g. catamenial seizures) and the striking differences in terms of sex distribution observed in some syndromes.

## 4. Epilepsy with eyelid myoclonia as a spectrum disorder: clinical, genetic and prognostic implications

In this thesis, we thoroughly delineated the electroclinical features and prognostic factors in a large cohort of patients with EEM, by means of the largest cohort of patients ever reported.

Through the use of modern clustering techniques, we provided evidence that EEM could be considered a spectrum of disease subtypes, with electroclinical and prognostic implications. In the first study, we highlighted the existence of two different sub-phenotypes equally distributed in our cohort, namely EEM-only and EEM-plus, which differ to a great extent in terms of seizure outcome and neuropsychiatric profile (Cerulli Irelli et al., 2022b). Our multidimensional classification of EEM patients could greatly help clinicians in the prognostic profiling and in the early identification of more complex patients. For instance, when dealing with a probable EEM-plus patient, characterized by early age at epilepsy onset, intellectual disability, self-induced seizures, EM status epilepticus and atypical EEG patterns, the chance of achieving seizure control during a long-term follow-up could be considered low and the patient should be addressed early to specialized epilepsy centers. Conversely, in patients with the EEM-only sub-phenotype, characterized by later age at onset, normal intellectual functioning, absence of psychiatric comorbid conditions and status epilepticus, a favourable epilepsy course could be expected. Our observation shed new light on the long-term seizure outcome of this syndrome, considering that previous literature almost invariably described an unfavourable prognosis in EEM (Giuliano et al., 2019; Covanis, 2015), independently of the electroclinical features observed. The recognition of distinct sub-phenotypes within the spectrum of EEM could therefore help clinicians to differentiate patients based on

selected clinical features, allowing a more personalized approach based on expected disease severity.

In the second research we conducted (Cerulli Irelli et al., 2022d), we expanded the initial cohort by also including EEM patients with occasional myoclonia over body districts other than eyelids, previously considered to display an intermediate phenotype between EEM and JME. Our data provided evidence that these patients should be more properly classified within the EEM spectrum, in spite of some distinctive features. Moreover, we highlighted the impact of age at epilepsy onset on the clinical variability observed in EEM. If previous studies showed age at disease onset as a key factor to distinguish relevant disease subtypes in several medical diseases, with genetic, clinical, prognostic and therapeutic implications (Bellivier et al., 2001; Naj et al., 2014), our approach represented a novelty in epilepsy research. Through the use of unidimensional clustering techniques (i.e., based uniquely on age at onset), we highlighted that an age at onset-based classification of the EEM spectrum revealed three relevant disease subtypes characterized by important electroclinical differences: an early-onset, an intermediate-onset and a late-onset subgroup. Interestingly, the early-onset cluster greatly overlapped with the previously described "EEM-plus" subgroup, with respect to its neuropsychiatric profile and seizure outcome, whereas the intermediate onset cluster was related to the above-mentioned "EEM-only", considering its "pure" phenotype and the favorable response to ASMs. In addition, a late-onset subgroup was identified, more closely overlapping some JME characteristics.

Furthermore, our age at onset-based classification revealed some interesting findings which may pave the way for future genetic studies in EEM considering the identified subtypes. Indeed, we showed a significant inverse association between the probability of having a family history of epilepsy and the age at seizure onset, providing clinical

evidence of a more prominent role of *de novo* mutations in earliest onset cases. Conversely, later onset patients showed a higher probability of having relatives affected by EEM, suggesting a more relevant role of complex inherited mechanisms in these patients, as observed in the majority of GGE syndromes (Marini et al., 2004). Our results suggest that earliest onset patients (especially in case of intellectual disability and EMA-plus characteristics) may be more suitable to be investigated with single-gene approaches to identify new monogenic variants, both in clinical and research setting. Moreover, we believe that our innovative age at onset-based classification of EEM patients should be replicated in large cohort studies including all GGE syndromes. Indeed, age at onset has been increasingly considered as a crucial factor in the classification of epilepsies (Specchio et al., 2022; Zuberi et al., 2022; Riney et al., 2022), and modern clustering techniques may be used to identify relevant age at onset-based subgroups within the context of GGE in terms of prognosis, electroclinical and genetic features.

A striking female preponderance has been always observed in all EEM subtypes and has been therefore considered as an important disease hallmark in this clinical entity (Covanis et al., 2015; Striano et al., 2009). Although the degree of female preponderance observed in EEM exceeded that observed in other female-preponderant GGE syndromes (mainly JME), no study previously provided a sex-based stratification of EEM patients. In our third study, female EEM patients showed a higher prevalence of several clinical markers of occipital cortex hyperexcitability (namely eye closure/photosensitivity persistence at the last follow-up visit and a history of migraine), which has been previously considered as an important factor in the pathophysiology of this syndrome (Vaudano et al., 2014). The hypothesized sexual dimorphism in occipital cortex excitability may also help to explain the female preponderance observed along the entire EEM continuum. Future

transcranial magnetic stimulation studies should address this topic, systematically evaluating the excitability of the occipital cortex in men and women with EEM.

In this thesis, we also provided for the first-time evidence about treatment withdrawal outcome in EEM (Cerulli Irelli et al., 2022a and 2022d), documenting a high rate of seizure relapse in all identified EEM sub-phenotypes. In this setting, if on the one hand we were able to identify several variables and specific disease subtypes capable of early predicting sustained remission during the long-term, on the other we showed that a life-long epilepsy could be expected in almost the entire EEM continuum, as observed in JME (Martinez-Juarez et al., 2006).

In conclusion, our research sheds new light on the electroclinical spectrum of EEM and provides relevant clinical data which may help clinicians in a better prognostic profiling and classification of these patients. Moreover, our observations pave the way for future genetic, neuroimaging and neurophysiological studies which may further contribute to delineate this generalized epilepsy syndrome.

## 5. Tables

| Table 1. Main clinical characteristics of idiopathic generalized epilepsies | | | | |
|---|---|---|---|---|
| | **JME** | **GTCA** | **CAE** | **JAE** |
| Age at onset | | | | |
| Usual | 10–24 years | 10–25 years | 4–10 years | 9–13 years |
| Range | 8–40 years | 5–40 years | 2–13 years<br><br>Caution if diagnosing at <4 years of age | 8–20 years<br><br>Exceptional cases may present in adulthood |
| Development | Typically normal, but may have learning disorder or ADHD | Typically normal, but may have learning disorder or ADHD | Typically normal, but may have learning difficulties or ADHD | Typically normal, but may have learning difficulties or ADHD |
| Main seizure type | Myoclonic seizures, seen predominantly on awakening | Generalized tonic–clonic seizures typically within 2 h of awakening | Absences: at least daily to multiple per day, with a typical duration of 3-20 seconds | Absences: usually less than daily, during 5-30 seconds, less complete loss of awareness |
| Other seizure types | 1) Febrile seizures in approximately 4%–5%<br><br>2) Generalized tonic–clonic seizures in >90%, which are often preceded by myoclonic jerks (myoclonic–tonic–clonic), | 1) Febrile seizures in approximately 15%<br><br>Other seizure types (e.g. absences and myoclonic jerks) must not be present to confirm this diagnosis | 1) Febrile seizures: occasional<br><br>2) GTCS rarely precede or occur during period of frequent absences but may occur later with evolution to other IGE syndrome | 1) Febrile seizures: occasional<br><br>2) GTCS may precede and commonly occur during the period of frequent absences |

| Table 1. Main clinical characteristics of idiopathic generalized epilepsies | | | | |
|---|---|---|---|---|
| | **JME** | **GTCA** | **CAE** | **JAE** |
| | and often occur on awakening<br><br>3) Absence seizures in 33%, typically brief (3–8 s), infrequent (<daily) | | | |
| Triggers | Sleep deprivation<br><br>Photic stimulation | Sleep deprivation | | |
| EEG background | Normal | Normal | | |
| Epileptiform discharges | Irregular, generalized 3–5.5-Hz spike-wave and polyspike-wave seen in all states<br><br>May fragment in sleep | Generalized 3–5.5-Hz spike-wave or polyspike-wave, which may be seen only in sleep<br><br>May fragment in sleep | OIRDA in 21% | Normal |
| Photoparoxsmal response | Seen in 30%–90% and may trigger myoclonic jerks or generalized myoclonic–tonic–clonic seizures | May be seen | Rare<br>IPS triggers generalized spike-wave in 15%–21% but does not induce seizures | Rare<br>IPS triggers generalized spike-wave in 25% but does not induce seizures |
| Hypeventilation induction | 33% have hyperventilation-induced generalized spike-wave discharge but rarely induces absence seizures | May be seen | 87% | 87% |

**Table 1. Main clinical characteristics of idiopathic generalized epilepsies**

| | JME | GTCA | CAE | JAE |
|---|---|---|---|---|
| Ictal EEG | Disorganized discharges significantly more common with absences in JME than CAE<br><br>Generalized polyspike-wave with myoclonic jerks<br><br>3.5–6-Hz generalized spike-wave or polyspike-wave with absences<br><br>Generalized spikes with tonic phase of generalized tonic–clonic seizure followed by spike-wave during clonic phase, but often obscured by muscle artifact | Generalized spikes with tonic phase followed by spike-wave during clonic phase, but often obscured by muscle artifact | Regular 3-Hz (range = 2.5–4 Hz) generalized spike-wave; 21% may have absences starting at 2.5-Hz spike-wave, and 43% may have absences starting at 4 Hz; if no generalized spike-wave is seen with hyperventilation for 3 min in an untreated patient, CAE can be excluded.<br><br>Disorganized discharges less frequent | Regular 3–5.5-Hz generalized spike-wave<br><br>If no generalized spike-wave is seen with hyperventilation for 3 min in an untreated patient, JAE can be excluded<br><br>Disorganized discharges 8 times more frequent than CAE |

Abbreviations: ADHD = attention deficit hyperactivity disorder; CAE= childhood absence epilepsy; GTCA = generalized tonic-clonic seizures alone syndrome; GTCS = generalized tonic-clonic seizures; JAE = juvenile absence epilepsy; JME = juvenile myoclonic epilepsy; OIRDA = occipital intermittent rhythmic delta activity.

| **Table 2. Demographic and clinical characteristics** | |
|---|---|
| Age, years, median (IQR) | 22 (17-32) |
| Sex, female, n (%) | 123 (71.5) |
| Age at epilepsy onset (IQR) | 7 (5-10) |
| Follow-up duration, years, median (IQR) | 14 (8.25-23.75) |
| History of FS, n, % | 19 (11) |
| Family history of epilepsy in a 1st degree relative, n (%) | 35 (20.3) |
| Family history of epilepsy in a 2nd degree relative, n (%) | 21 (12.2) |
| Family history of epilepsy in a 3rd degree relative, n (%) | 13 (7.6) |
| Family history of FS in a 1st or 2nd degree relative, n (%) | 6 (3.5) |
| Psychiatric comorbidities, n (%) | 45 (26.2) |
| Borderline IF or mild ID, n (%) | 55 (32) |
| Abbreviations: ID = intellectual disability ; FS = febrile seizures ; IF = intellectual functioning; IQR = interquartile range; SD = standard deviation | |

**Table 3. Predictors of relapse and no-remission patterns, as determined by multivariable multinomial logistic regression**

| | Relapse | | | No remission | | |
|---|---|---|---|---|---|---|
| Predictor | OR | 95% CI | p-value | OR | 95% CI | p-value |
| Male sex | 0.5 | 0.17-1.41 | 0.2 | 0.68 | 0.26-1.79 | 0.4 |
| Early-onset epilepsy | 2.42 | 0.91-6.41 | 0.08 | 4.88 | 1.82-12.98 | 0.002* |
| Follow-up duration | 1.04 | 1.01-1.08 | 0.02 | 0.98 | 0.94-1.02 | 0.3 |
| Febrile seizures | 3.6 | 0.55-23.25 | 0.2 | 9.01 | 1.67-47.61 | 0.01* |
| Family history of epilepsy in a 1st or 2nd degree relative | 3.11 | 1.22-7.94 | 0.02* | 1.29 | 0.51-3.27 | 0.6 |
| Borderline IF or mild ID | 2.05 | 0.73-5.78 | 0.2 | 1.06 | 0.39-2.87 | 0.9 |
| Psychiatric comorbidities | 1.73 | 0.58-5.18 | 0.3 | 2.28 | 0.81-6.41 | 0.1 |
| Eyelid myoclonic status epilepticus | 1.86 | 0.37-9.43 | 0.5 | 5.05 | 1.24-20.8 | 0.02* |
| Self-induced seizures | 0.72 | 0.11-4.48 | 0.7 | 2.39 | 0.55-10.41 | 0.2 |
| History of GTCS | 3.15 | 1.05-9.43 | 0.04* | 2.19 | 0.85-5.61 | 0.1 |
| History of both ECS and PS | 1.71 | 0.64-4.57 | 0.3 | 1.65 | 0.64-4.22 | 0.3 |
| EEG focal abnormalities | 1.83 | 0.69-4.85 | 0.2 | 1.68 | 0.63-4.08 | 0.3 |

Note: Data are presented as odds ratios (ORs) along with 95% confidence intervals (CIs). The asterisks indicate statistically significant variables ($p<0.05$). Abbreviations: ECS = eye closure sensitivity; GTCS = generalized tonic-clonic seizures; ID = intellectual disability; IF = intellectual functioning; PS = photosensitivity

**Table 4. Prognostic factors for sustained terminal remission according to multivariable Cox proportional hazards model**

| Predictors | HR | 95% CI | p-value |
|---|---|---|---|
| Female sex | 0.81 | 0.46-1.44 | 0.5 |
| Early onset of epilepsy | 0.41 | 0.24-0.7 | <0.001* |
| History of FS | 0.17 | 0.05-0.76 | 0.02* |
| Family history of epilepsy in a 1st or 2nd degree relative | 0.72 | 0.42-1.21 | 0.2 |
| Borderline IF or mild ID | 0.95 | 0.53-1.72 | 0.9 |
| Psychiatric comorbidities | 0.34 | 0.16-0.71 | 0.004* |
| Eyelid myoclonic status epilepticus | 0.54 | 0.22-1.28 | 0.2 |
| Self-induced seizures | 0.71 | 0.25-2.04 | 0.5 |
| GTCS during history | 0.47 | 0.27-0.82 | 0.008* |
| History of both PS and ECS | 1.15 | 0.65-2.02 | 0.6 |
| EEG focal abnormalities | 0.97 | 0.56-1.68 | 0.9 |

Note: Data are presented as hazard ratios (HRs) along with 95% confidence intervals (CIs). The asterisks indicate statistically significant variables ($p < .05$). Abbreviations: ECS = eye closure sensitivity; EM = eyelid myoclonia; FS = febrile seizures; GTCS = generalized tonic-clonic seizures; ID = intellectual disability; IF = intellectual functioning; PS = photosensitivity; STR = sustained terminal remission

**Table 5. Patient clinical characteristics stratified according to cluster**

| Variable | Cluster 1 (86 pts) | Cluster 2 (86 pts) | p-value |
|---|---|---|---|
| Sex, female, n (%) | 62 (72.1) | 61 (70.9) | 1 |
| Early epilepsy onset, n (%) | 39 (45.3) | 70 (81.4) | <0.001* |
| Age of epilepsy onset, years, median (IQR) | 9 (6-10.7) | 6 (4-8) | <0.001* |
| Follow-up duration, years, median (IQR) | 15 (8-25.7) | 14 (9.2-21) | 0.46 |
| History of FS, n, % | 5 (5.8) | 14 (16.3) | 0.049* |
| Family history of epilepsy in a 1st or 2nd degree relative, n (%) | 30 (34.9) | 27 (31.4) | 0.7 |
| Psychiatric comorbidities, n (%) | 18 (20.9) | 27 (31.4) | 0.16 |
| Borderline IF or mild ID, n (%) | 14 (16.3) | 41 (47.7) | <0.001* |
| Eyelid myoclonic status epilepticus, n (%) | 4 (4.6) | 18 (20.9) | 0.002* |
| Self-induced seizures, n (%) | 4 (4.6) | 13 (15.1) | 0.03* |
| History of GTCS, n (%) | 61 (70.9) | 59 (68.6) | 0.87 |
| History of both PS and ECS, n (%) | 51 (59.3) | 66 (76.7) | 0.02* |
| Focal EEG abnormalities | 27 (31.4) | 30 (34.9) | 0.75 |
| Remission pattern, n (%) | 59 (68.6) | 0 | <0.001* |
| Relapsing pattern, n (%) | 27 (31.4) | 20 (23.3) | 0.3 |
| No remission pattern, n (%) | 0 | 66 (76.7) | <0.001* |

Abbreviations: ECS = eye closure sensitivity; EM = eyelid myoclonia; FS = febrile seizures; ID = intellectual disability; IF = intellectual functioning; IQR = interquartile range; PS = photosensitivity. Note: the asterisks indicate statistically significant variables ($p<0.05$)

**Table 6. Clinical characteristics according to age at onset subgroup**

| | EO-EEM (118 pts) | IO-EEM (87 pts) | LO-EEM (62 pts) | p value |
|---|---|---|---|---|
| Sex, female (%) | 89 (75.4) | 61 (70.1) | 45 (72.6) | 0.7 |
| Age at epilepsy onset, years, median (IQR) | 5 (3-6) | 9 (7-9) | 13 (11.7-14) | <0.001* |
| Follow-up duration, years, median (IQR) | 16 (11-24) | 13 (8-24) | 13 (6.8-22) | 0.28 |
| Age at the last follow-up visit, median (IQR) | 21 (14-29) | 22 (17-32) | 24 (18-34) | 0.01* |
| Family history of epilepsy in 1st or 2nd degree relatives, n (%) | 27 (22.9) | 37 (42.5) | 19 (30.6) | 0.01* |
| Family history of EEM, n (%) | 2 (1.7) | 9 (10.3) | 5 (8.1) | 0.02* |
| Family history of febrile seizures, n (%) | 12 (10.2) | 8 (9.2) | 3 (4.8) | 0.5 |
| History of febrile seizures in 1st and 2nd degree relatives, n (%) | 16 (13.7) | 8 (9.2) | 6 (9.7) | 0.5 |
| Borderline intellectual functioning, n (%) | 26 (22) | 13 (14.9) | 8 (12.9) | 0.2 |
| Mild intellectual disability, n (%) | 24 (20.3) | 6 (6.9) | 3 (4.8) | 0.002* |
| Migraine with/without aura, n (%) | 13 (11) | 10 (11.5) | 14 (22.6) | 0.08 |
| Psychiatric comorbidities, n (%) | 37 (31.6) | 13 (13.1) | 14 (22.6) | 0.009* |
| Mood disorders, n (%) | 14 (11.9) | 5 (5.7) | 9 (14.5) | 0.2 |
| Behavioral disorders, n (%) | 20 (16.9) | 6 (6.9) | 5 (8.1) | 0.052 |
| Psychotic disorder, n (%) | 3 (2.5) | 1 (1.1) | 0 | 0.4 |
| Seizure types | | | | |
| Generalized tonic-clonic seizures, n (%) | 70 (59.3) | 61 (70.1) | 51 (82.3) | 0.006* |
| Myoclonia in body districts other than eyelids, n (%) | 20 (16.9) | 17 (19.5) | 21 (33.9) | 0.03* |
| Eyelid myoclonia status epilepticus, n (%) | 16 (13.5) | 10 (11.6) | 9 (14.5) | 0.8 |
| Self-induced seizures, n (%) | 23 (19.5) | 15 (17.2) | 10 (16.1) | 0.8 |
| Catamenial worsening of seizures, n (%) | 10 (11.2) | 6 (9.8) | 7 (15.6) | 0.6 |
| EEG features | | | | |
| ECS at any time during follow-up, n (%) | 89 (75.4) | 68 (78.2) | 50 (80.6) | 0.7 |
| PS at any time during follow-up, n (%) | 110 (93.2) | 80 (92) | 55 (88.7) | 0.6 |
| ECS at the last follow-up visit, n (%) | 44 (45.4) | 35 (40.2) | 22 (35.5) | 0.8 |
| PS at the last follow-up visit, n (%) | 62 (52.5) | 42 (48.3) | 22 (35.5) | 0.04* |
| Polyspike-wave discharges, n (%) | 93 (78.8) | 61 (70.9) | 44 (73.3) | 0.4 |
| Focal spikes, n (%) | 17 (17.2) | 15 (20.5) | 9 (20.5) | 0.8 |
| Seizure outcome | | | | |
| ASM refractoriness, n (%) | 75 (63.6) | 41 (47.1) | 31 (50) | 0.04* |
| ≥ 2 ASMs used at the last visit, n (%) | 60 (50.8) | 30 (34.5) | 27 (43.5) | 0.06 |
| 2-year remission during history, n (%) | 68 (57.6) | 55 (63.2) | 35 (72.6) | 0.1 |

Abbreviations : ASM = antiseizure medication ; ECS = eye closure sensitivity ; EEM = epilepsy with eyelid myoclonia ; EO = early onset ; IO = intermediate onset ; LO = late-onset ; PS = photosensitivity. Note : The asterisks indicate statistically significant variables ($p$<0.05).

**Table 7. Comparison of clinical and EEG characteristics according to the presence or not of sporadic myoclonia over body regions other than eyelids**

| | Body-MYO (58 pts) | No-Body-MYO (209 pts) | p value |
|---|---|---|---|
| Sex, female (%) | 45 (77.6) | 150 (71.8) | 0.4 |
| Age at epilepsy onset, years, median (IQR) | 8.5 (6-13) | 7 (5-10) | 0.02* |
| Follow-up duration, years, median (IQR) | 15.5 (10.7-26) | 14 (8-23) | 0.1 |
| Age at the last follow-up visit, median (IQR) | 24 (18-33) | 21 (16-30) | 0.04* |
| Family history of epilepsy in 1st or 2nd degree relatives, n (%) | 19 (32.8) | 64 (30.6) | 0.7 |
| Family history of EEM, n (%) | 5 (8.6) | 11 (5.3) | 0.4 |
| Family history of JME, n (%) | 3 (5.2) | 4 (1.9) | 0.2 |
| History of febrile seizures in 1st or 2nd degree relatives, n (%) | 8 (13.8) | 15 (7.2) | 0.1 |
| Personal history of febrile seizures, n (%) | 7 (12.3) | 23 (11) | 0.8 |
| Borderline intellectual functioning, n (%) | | | |
| Mild intellectual disability, n (%) | 11 (19) | 22 (10.5) | 0.08 |
| Migraine with or without aura, n (%) | 16 (27.6) | 21 (10) | <0.001* |
| Psychiatric comorbidities, n (%) | 13 (22.8) | 49 (23.8) | 0.9 |
| Mood disorders, n (%) | 8 (13.8) | 21 (10) | 0.5 |
| Behavioral disorders, n (%) | 5 (8.6) | 24 (11.5) | 0.6 |
| Psychotic disorder, n (%) | 0 | 4 (1.9) | 0.6 |
| Seizure types | | | |
| Generalized tonic-clonic seizures, n (%) | 49 (84.5) | 133 (63.6) | 0.002* |
| Eyelid myoclonia status epilepticus, n (%) | 7 (12.1) | 28 (13.7) | 0.8 |
| Self-induced seizures, n (%) | 10 (17.2) | 38 (18.2) | 0.9 |
| Catamenial worsening of seizures, n (%) | 7 (15.6) | 16 (10.7) | 0.4 |
| EEG features | | | |
| ECS at any time during follow-up, n (%) | 46 (79.3) | 161 (77) | 0.7 |
| PS at any time during follow-up, n (%) | 55 (94.8) | 190 (90.9) | 0.4 |
| ECS at the last follow-up visit, n (%) | 21 (36.2) | 80 (38.3) | 0.9 |
| PS at the last follow-up visit, n (%) | 27 (46.5) | 99 (47.4) | 1 |
| Polyspike-wave discharges, n (%) | 36 (62.1) | 156 (75.7) | 0.04* |
| Focal spikes, n (%) | 15 (25.9) | 39 (18.7) | 0.2 |
| Seizure outcome | | | |
| ASM refractoriness, n (%) | 34 (58.6) | 113 (54.1) | 0.6 |
| 2-year remission during history, n (%) | 38 (65.5) | 130 (62.2) | 0.7 |
| ASM withdrawal attempt, n (%) | 21 (36.2) | 73 (34.9) | 0.9 |
| Seizure recurrence after ASM withdrawal, n (%) | 17 (77.3) | 54 (75) | 0.8 |

Abbreviations : ASM = antiseizure medication ; ECS = eye closure sensitivity ; EEM = Epilepsy with eyelid myoclonia ; EO = early onset ; IO = intermediate onset ; LO = late-onset ; PS = photosensitivity. Note : The asterisks indicate statistically significant variables ($p<0.05$).

| **Table 8. Comparison of demographic and clinical characteristics according to sex** | | | |
|---|---|---|---|
| | Females (195 patients) | Males (72 patients) | p value |
| Age at epilepsy onset, years, median (IQR) | 7 (5-10) | 8 (5-10) | 1 |
| Follow-up duration, years, median (IQR) | 14 (9-24) | 14 (7.2-23.8) | 0.8 |
| Age at the last follow-up visit, median (IQR) | 22 (17-31) | 21 (17-31.7) | 0.4 |
| Family history of epilepsy in 1st or 2nd degree relatives, n (%) | 57 (29.2) | 26 (36.1) | 0.3 |
| Personal history of febrile seizures, n (%) | 22 (11.2) | 8 (11.1) | 1 |
| Borderline intellectual functioning and or mild intellectual disability, n (%) | 51 (26.2) | 29 (40.3) | 0.025* |
| Migraine with or without aura, n (%) | 32 (16.4) | 5 (6.9) | 0.047* |
| Psychiatric comorbidities, n (%) | 44 (22.6) | 18 (25) | 0.7 |
| Seizure types experienced during history | | | |
| Generalized tonic-clonic seizures, n (%) | 133 (68.2) | 49 (68.1) | 1 |
| Myoclonia in body districts other than eyelid, n (%) | 45 (23.1) | 13 (18.1) | 0.4 |
| Eyelid myoclonia status epilepticus, n (%) | 26 (13.5) | 9 (12.7) | 0.8 |
| Provoking factors | | | |
| Self-induced seizures, n (%) | 35 (17.9) | 13 (18.1) | 1 |
| Catamenial worsening of seizures, n (%) | 23 (11.8) | 0 | |
| ECS at any time during follow-up, n (%) | 156 (80) | 51 (70.8) | 0.13 |
| PS at any time during follow-up, n (%) | 66 (91.7) | 66 (91.8) | 1 |
| ECS persistence at the last visit, n (%) | 82 (48.8) | 19 (30.2) | 0.02* |
| PS persistence at the last visit, n (%) | 109 (60.2) | 19 (31.1) | <0.001* |
| EEG features | | | |
| Polyspike-wave discharges, n (%) | 140 (71.8) | 52 (72.2) | 0.9 |
| Focal spikes, n (%) | 38 (19.5) | 16 (22.2) | 0.6 |
| ASM treatment | | | |
| ASM tried during history, median (IQR) | 2 (2-4) | 3 (2-4) | 0.4 |
| Valproate use at the last visit, n (%) | 92 (47.2) | 54 (75) | <0.001* |
| Levetiracetam use at the last visit, n (%) | 77 (39.5) | 19 (26.4) | 0.048* |

Abbreviations : ASM = antiseizure medication ; ECS = eye closure sensitivity ; EEM = Epilepsy with eyelid myoclonia ; EO = early onset ; IO = intermediate onset ; LO = late-onset ; PS = photosensitivity. Note : The asterisks indicate statistically significant variables ($p<0.05$).

**Table 9. Multivariable logistic regression models for drug-resistance according to sex**

| **Multivariable model developed for female patients** | | | |
|---|---|---|---|
| Predictor | OR | 95% CI | p value |
| Age at epilepsy onset | 0.88 | 0.81-0.97 | 0.008* |
| Follow-up duration | 0.98 | 0.96-1.10 | 0.2 |
| Psychiatric comorbidities | 2.33 | 1.1-4.94 | 0.03* |
| Eyelid myoclonia status epilepticus | 2.78 | 1.12-6.94 | 0.03* |
| History of self-induced seizures | 2.29 | 0.99-5.31 | 0.053 |
| Catamenial worsening of seizures | 2.67 | 1.02-7.04 | 0.047* |
| **Multivariable model developed for male patients** | | | |
| Predictor | OR | 95% CI | p value |
| Follow-up duration | 1.01 | 0.97-1.04 | 0.8 |
| History of self-induced seizures | 2.31 | 0.63-8.51 | 0.2 |
| History of febrile seizures | 6.19 | 1.12-34.26 | 0.04* |

Abbreviations : CI = confidence interval ; OR = odd ratio. Note : The asterisks indicate statistically significant variables ($p<0.05$).

## 6. Figures

**Fig. 1 Prognostic factors of sustained terminal remission during follow-up**
Follow-up was truncated at 40 years and censored patients are indicated by crosses.

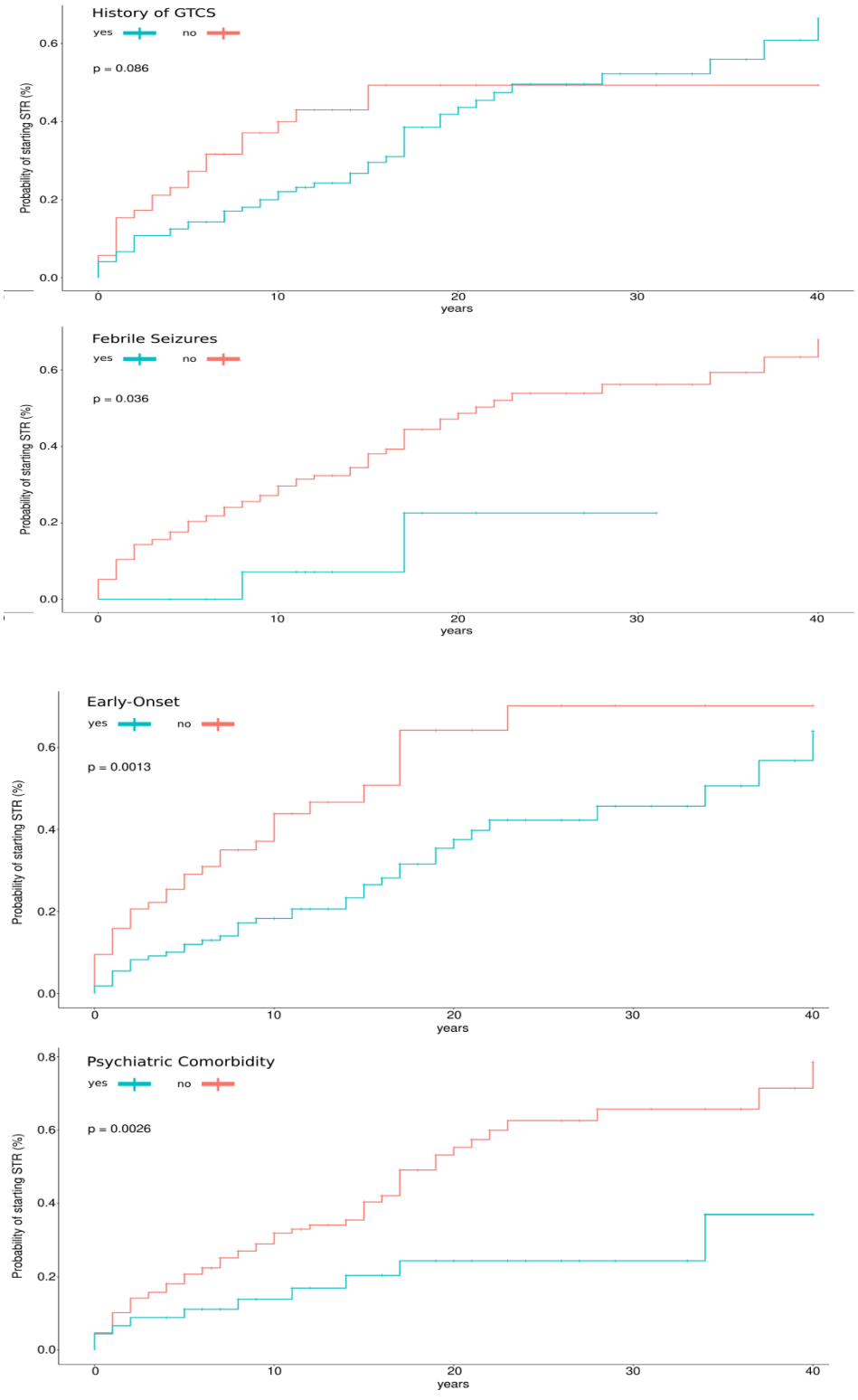


**Fig. 2 Electroclinical characteristics of clusters**

Radar plot showing the electroclinical differences between clusters. Cluster 1 refers to EMA-only patients, cluster 2 refers to EMA-plus patients. Abbreviations: ECS = eye closure sensitivity; EM = eyelid myoclonia; PS = photosensitivity

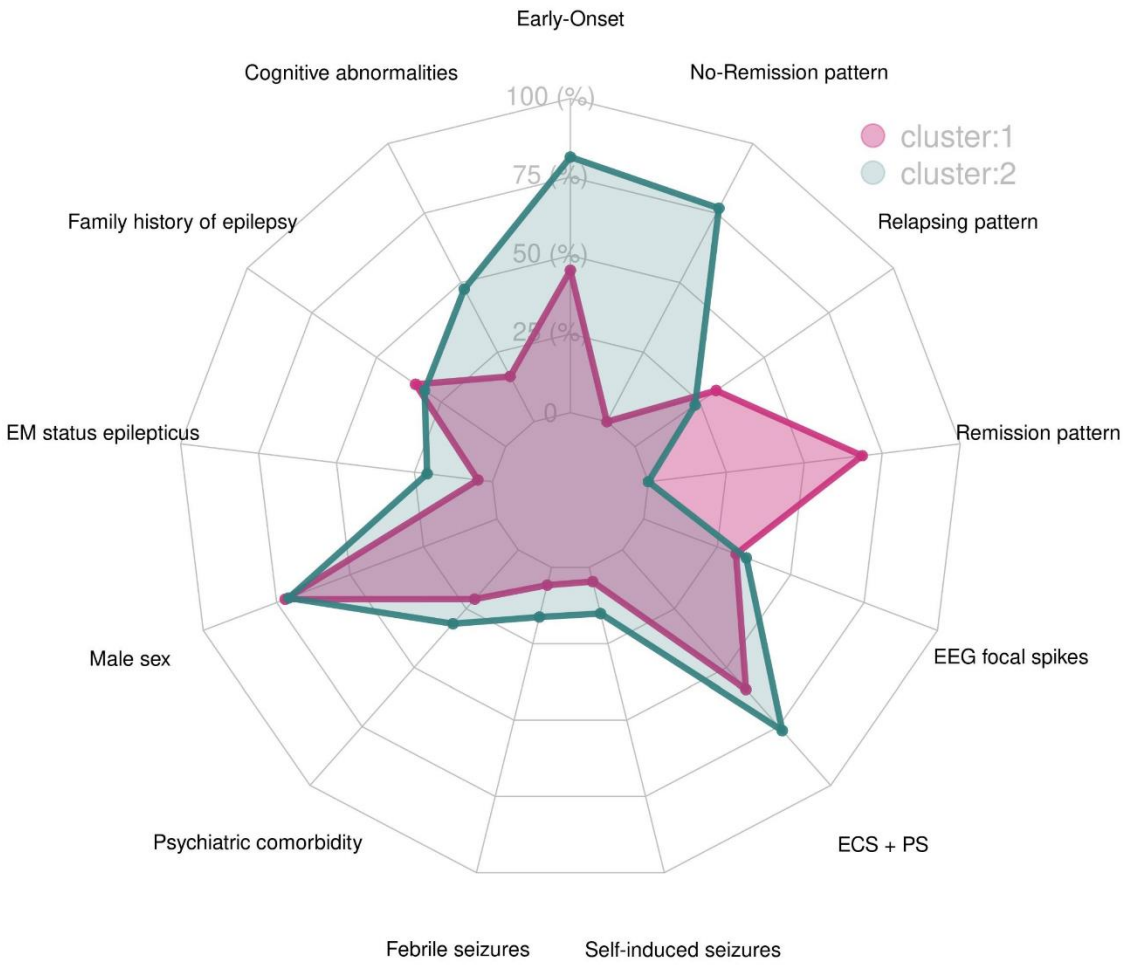


**Figure 3. Age at onset of each seizure type**

Body-MYO = myoclonia involving body districts other than eyelids; EM = eyelid myoclonia; GTCS = generalized tonic-clonic seizures

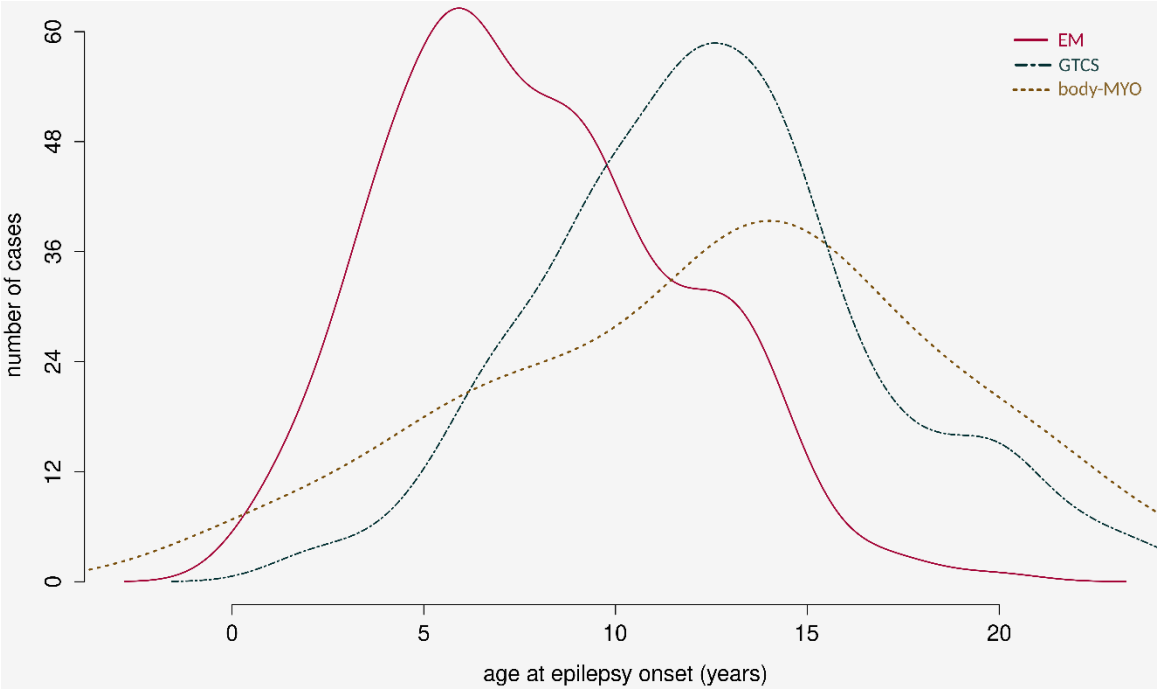


**Fig. 4 Distribution according to age at epilepsy onset and underlying clusters**

PANEL A: Kernel density estimation revealing three underlying modes according to age at epilepsy onset; PANEL B: Fisher-Jenks algorithm showing the optimal cut-off for patient classification into three distinct clusters (early, intermediate, late) according to age at epilepsy onset.

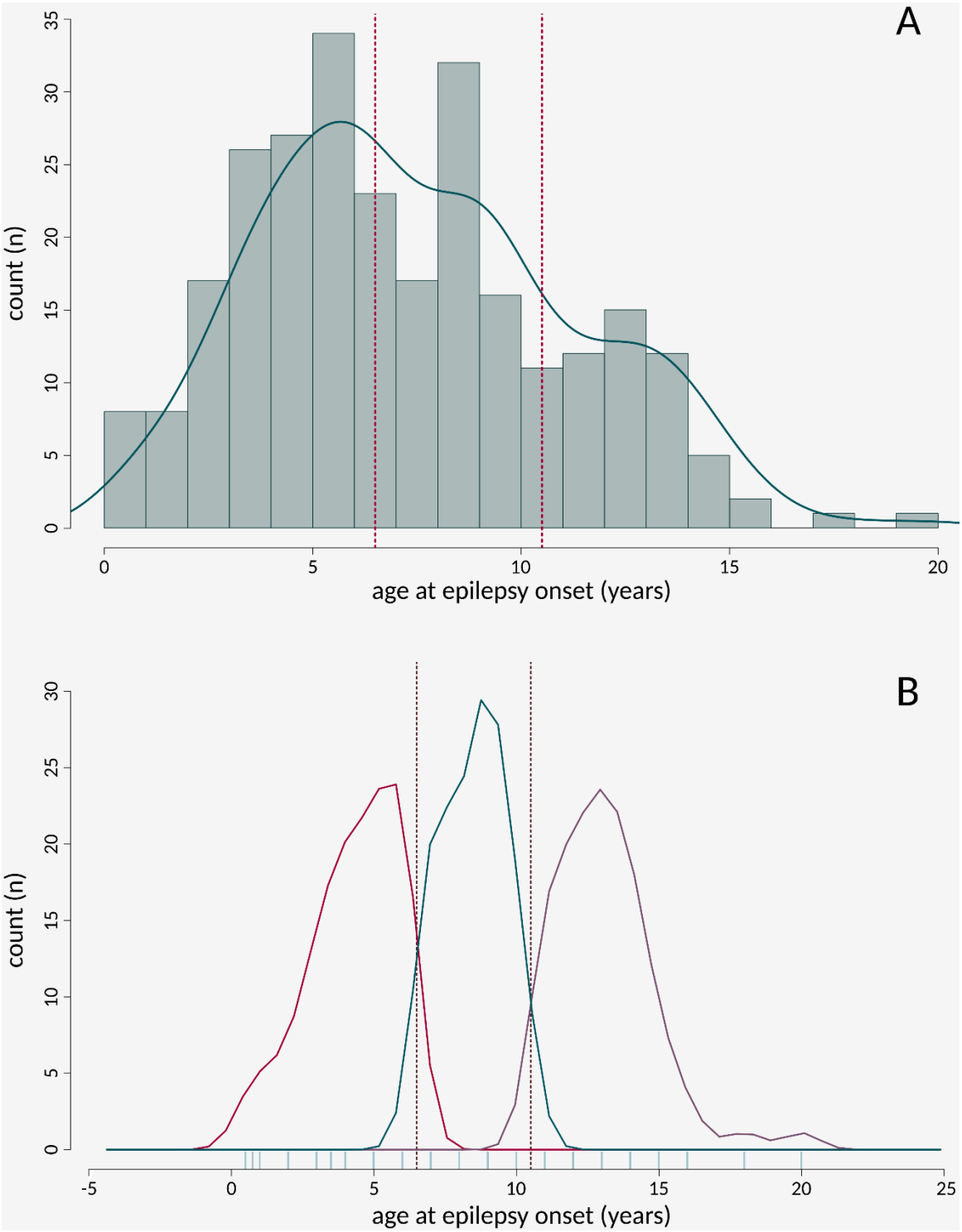
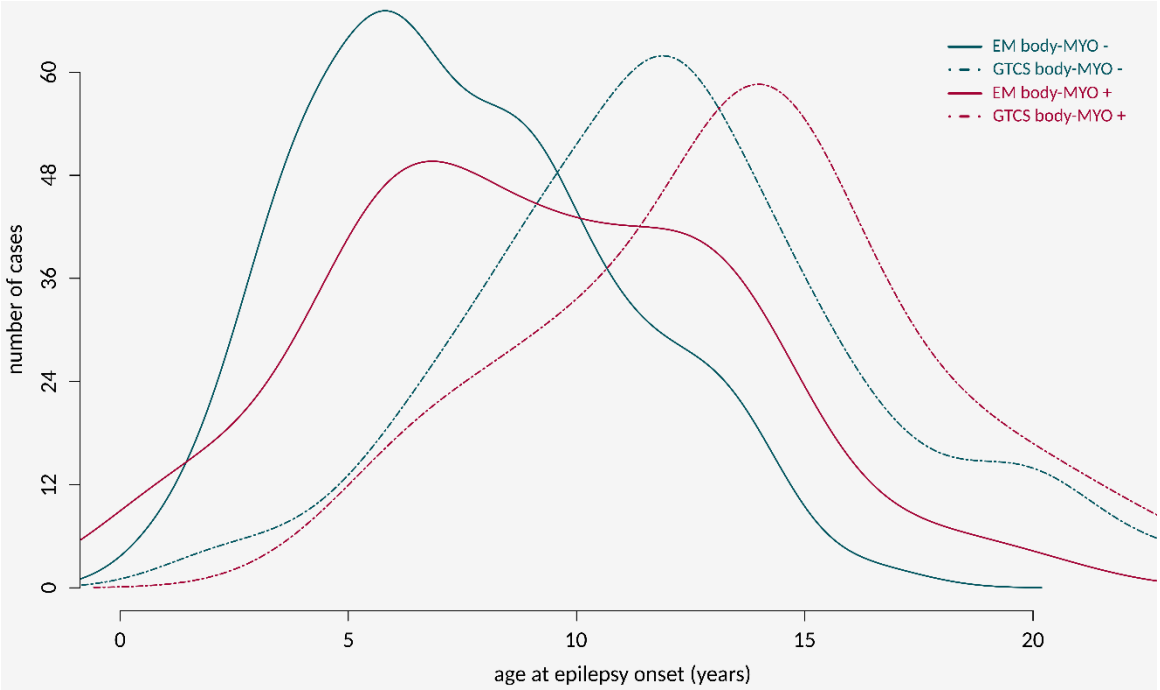


**Fig. 5 Age at onset of different seizure types in patients with sporadic myoclonia over body regions other than eyelids (body-MYO+) compared to the remaining cohort (body-MYO-)**

EM = eyelid myoclonia; GTCS = generalized tonic-clonic seizures.



## 7. Supplementary material

**Supplementary table 1. Prognostic factors for sustained terminal remission according to multivariable Cox proportional hazards model (using ag at onset as continuous variable)**

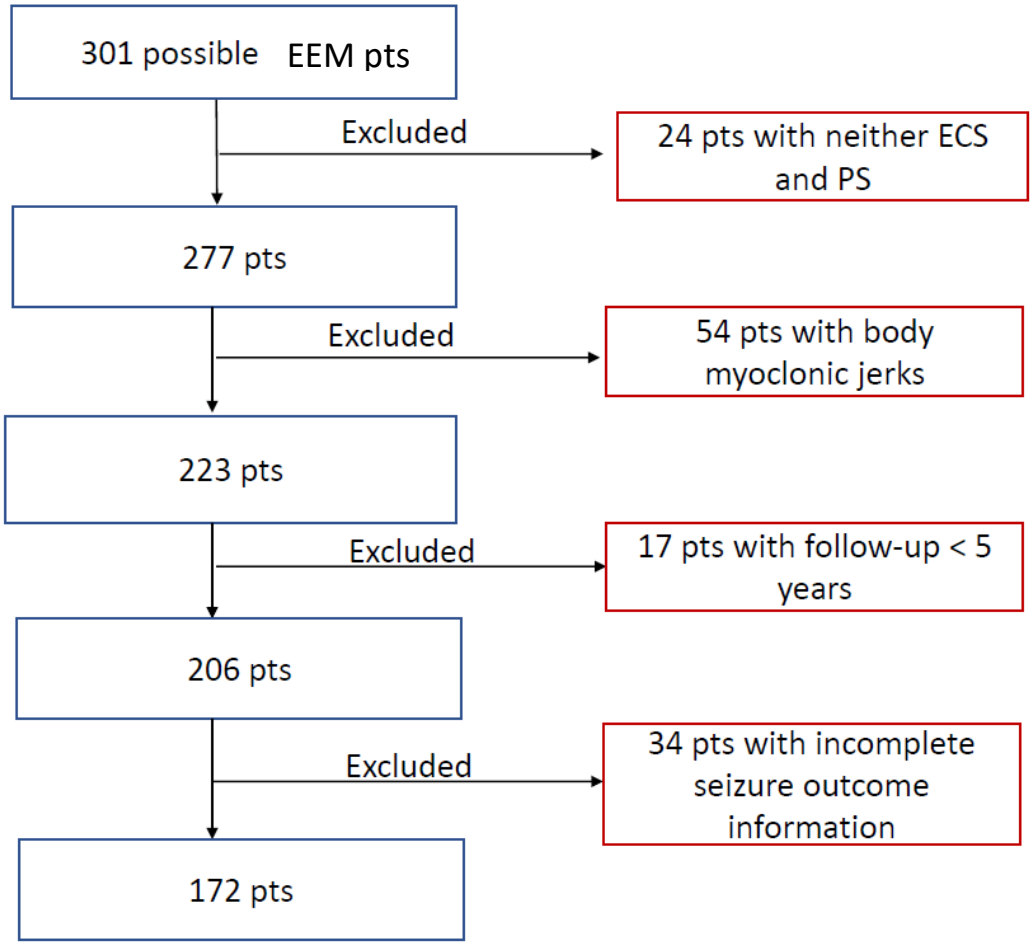
| Predictors | HR | 95% CI | p value |
|---|---|---|---|
| Female sex | 0.83 | 0.46-1.49 | 0.5 |
| Age at epilepsy onset (years) | 1.13 | 1.05-1.22 | 0.002* |
| History of FS | 0.22 | 0.05-0.9 | 0.04* |
| Family history of epilepsy in a 1st or 2nd degree relative | 0.72 | 0.42-1.23 | 0.2 |
| Borderline IF or mild ID | 0.85 | 0.47-1.54 | 0.6 |
| Psychiatric comorbidities | 0.39 | 0.19-0.8 | 0.01* |
| Eyelid myoclonic status epilepticus | 0.56 | 0.3-1.32 | 0.2 |
| Self-induced seizures | 0.63 | 0.22-1.77 | 0.4 |
| GTCS during history | 0.46 | 0.26-0.81 | 0.007* |
| History of both PS and ECS | 1.02 | 0.57-1.82 | 0.9 |
| EEG focal abnormalities | 1.01 | 0.58-1.75 | 1 |

Note: Data are presented as hazard ratios (HRs) along with 95% confidence intervals (CIs). The asterisks indicate statistically significant variables ($p < .05$). Abbreviations: ECS = eye closure sensitivity; EM = eyelid myoclonia; FS = febrile seizures; GTCS = generalized tonic-clonic seizures; ID = intellectual disability; IF = intellectual functioning; PS = photosensitivity; STR = sustained terminal remission

**Supplementary table 2. Linear regression results using as dependent variable the number of ASMs at the last follow-up visit**

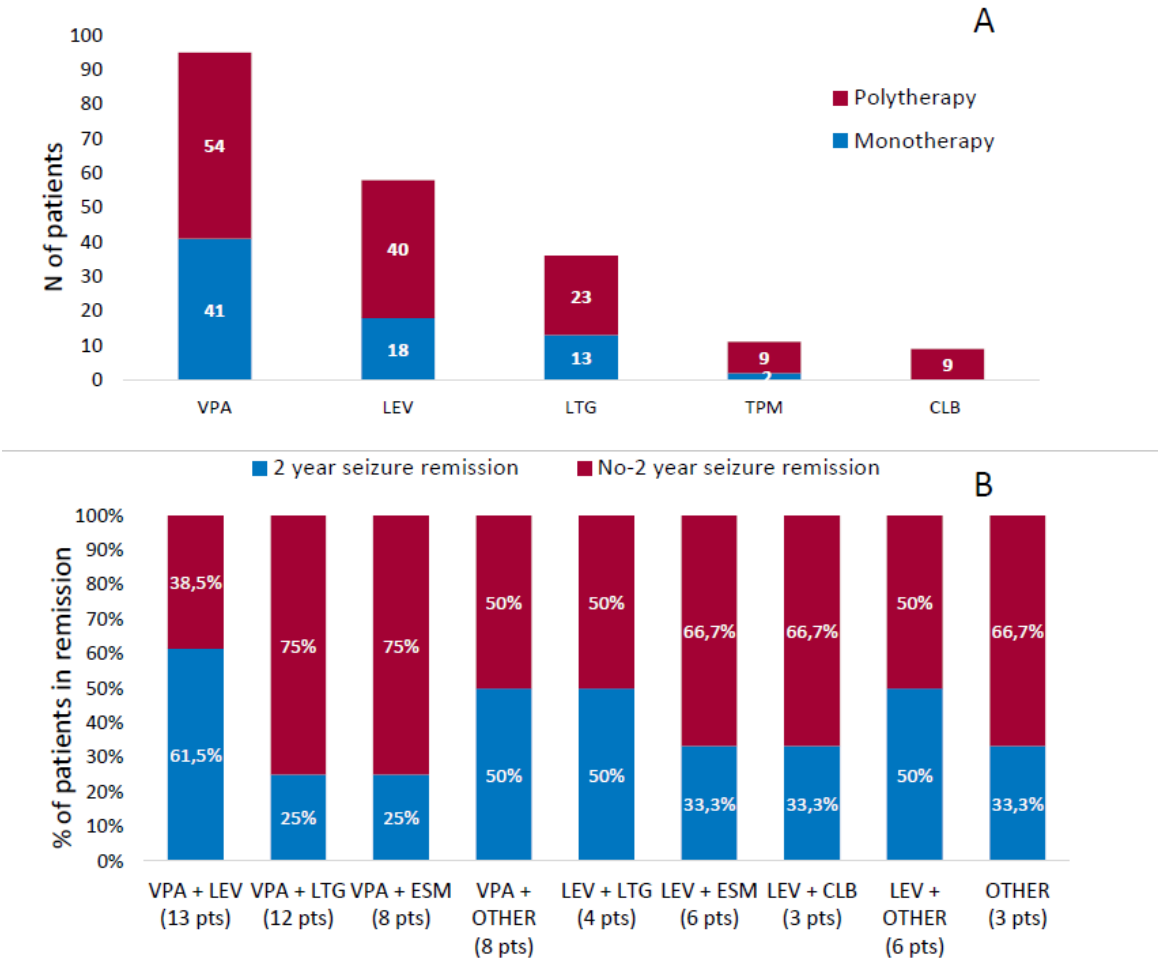
| | Univariable model | | Multivariable analysis | |
|---|---|---|---|---|
| Variables | β | p value | β | p value |
| Female sex | -0.14 | 0.06 | -0.12 | 0.1 |
| Early onset of epilepsy | 0.23 | 0.002* | 0.21 | 0.009* |
| Follow-up duration | 0.20 | 0.003* | 0.14 | 0.06 |
| Family history of epilepsy in 1st and 2nd degree | 0.07 | 0.35 | | |
| Borderline IF or mild ID | 0.12 | 0.09 | 0.04 | 0.64 |
| GTCS during history | 0.19 | 0.01* | 0.17 | 0.03* |
| History of both PS and ECS | 0.06 | 0.42 | | |
| EM status epilepticus during history | -0.05 | 0.51 | | |
| History of self-induced seizures | 0.11 | 0.14 | 0.13 | 0.08 |
| History of febrile seizures | 0.10 | 0.16 | 0.11 | 0.12 |
| EEG Focal spikes | 0.04 | 0.54 | | |

Abbreviations: ASM = Antiseizure medication; GTCS = generalized tonic-clonic seizures ; ID = Intellectual disability; IF = intellectual functioning, The asterisks indicate statistically significant variables ($p<0.05$)



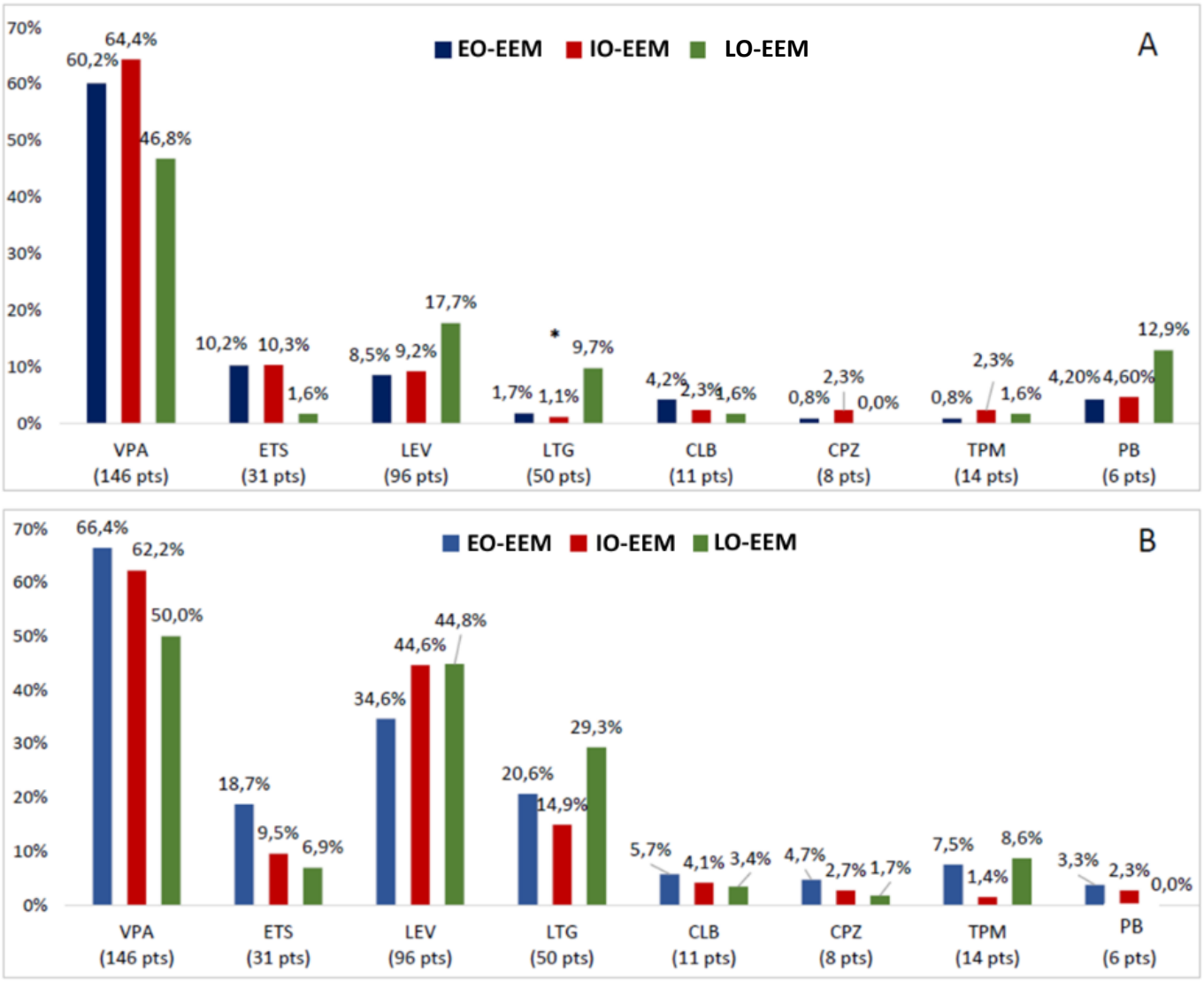

**Supplementary Figure 1. Patients' enrollment methods**

Abbreviations : ECS = Eye closure sensitivity ; EEM = epilepsy with eyelid myoclonia.




**Supplementary figure 2. Antiseizure medicatications use at the last follow-up visit**

The most common antiseizure medications(ASMs) used at last follow-up visit are represented in panel A. The number of patients using each ASM in monotherapy or in polytherapy regimens are shown in the bars.
The most common biotherapies used at last follow-up visit are shown in panel B. The percentage of patients experiencing 2 year remission at last follow-up visit for each bitherapy is expressed in the bar. Abbreviations: CLB = clobazam; ESM = ethosuximide; LEV = levetiracetam; LTG = lamotrigine; SR = seizureremission; TPM = topiramate; VPA = valproate




**Supplementary figure 3. Antiseizure medications used in different age at onset subgroups**

Panel A: ASMs used as first-line monotherapy. The number of patients using each ASM in the whole cohort is expressed in brackets. The percentage of patients using each ASM according to age subgroups is reported above the bars. Panel B: ASMs used at last observation : The number of patients using each ASM in the whole cohort is expressed in brackets. The percentage of patients using each ASM according to age subgroups is reported above the bars. Abbreviations: ASM = antiseizure medication; CLB = clobazam; CPZ = clonazepam; ETS = ethosuximide; LEV = levetiracetam; LTG = lamotrigine; PB = phenobarbital; TPM = topiramate. NOTE: the asterisk indicate statistically signficant variable

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