Original article

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Investigation of *SLC2A1* gene variants in genetic generalized epilepsy patients with eyelid myoclonia

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ABSTRACT – *Aims*. In addition to a complex inheritance pattern in genetic generalized epilepsy (GGE) syndromes, some studies have recently identified *SLC2A1* variants which lead to glucose transporter type 1 (*GLUT1*) defects, in patients diagnosed with GGE. Here, we investigated the possible role of *SLC2A1* variants in GGE patients with eyelid myoclonia (EM) which is a rare generalized seizure type associated with drug resistance and cognitive dysfunction.
Methods. After polymerase chain reaction with designed primers, sequencing of all *SLC2A1* exons was performed for 25 GGE-EM patients, as well as a control group of 15 GGE patients with absence seizures.
Results. Although various single nucleotide polymorphisms clustered in the ninth exon were detected, no variant was found in the two groups with GGE.
Conclusions. Even though the patient number in this study is small, the data suggest that *SLC2A1* variants do not play any causative role in GGE associated with EM.

Key words: genetic generalized epilepsy, eyelid myoclonia, *SLC2A1* variant, *GLUT1*

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Among genetic generalized epilepsy (GGE) syndromes, which mostly show a complex inheritance pattern, there are some rare monogenic causes underlying these disorders that present with different generalized seizure types. Recently, studies have shown *SLC2A1* variants, that encode glucose transporter type 1 (*GLUT1*), in GGE patients (Striano *et al*., 2012). The clinical spectrum associated with *SLC2A1* defects comprises developmental delay, movement disorders, cognitive problems, and antiepileptic drug-resistant seizures (Leen *et al*., 2010). Typically, early-onset absence seizures are reported in GGE patients with *SLC2A1* variants when epilepsy is prominent in the clinical picture. As the phenotypic spectrum associated with *SLC2A1* variants becomes broader, the same is true for the spectrum of epileptic

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disorders associated with *GLUT1* deficiency (Pong *et al*., 2012; Tzadok *et al*., 2014). The ketogenic diet could be the most appropriate solution for these disorders.

Eyelid myoclonia (EM) are a rare type of generalized seizures, characterized by fast jerking of the eyelids with slight rolling of the eyeballs, usually accompanied by brief absences with onset in childhood (Jeavons, 1977; Appleton *et al*., 1993). This specific seizure type is observed in patients with different aetiologies, such as genetic or symptomatic, but is mostly reported in patients with eyelid myoclonia with absences (EMA) (Capovilla *et al*., 2009; Caraballo *et al*., 2009). EMA syndrome consists of a triad of signs: frequent EM, eye closure sensitivity (ECS), and photosensitivity (PS). Although most of the patients with EMA are within normal cognitive range, borderline cognitive functioning may also be observed. The condition is known to be life-long and difficult to treat with antiepileptic drugs (Scuderi *et al*., 2000; Striano *et al*., 2002, 2009).

Given that *GLUT1* deficiency is reported as a cause of GGE, we aimed to examine our GGE patients with EM, as an endophenotype, for the possible presence of *SLC2A1* variants.

Methods

Patients

Twenty-five consecutive GGE patients with EM, followed in our tertiary epilepsy centre, were included in the study. Clinical and demographic characteristics, EEG findings, epilepsy syndromes, and treatments were recorded. Epilepsy syndromes were grouped according to ILAE criteria, based on clinical and diagnostic EEG features. EMA syndrome was defined as the concurrence of EM with/without absences and presence of both PS and ECS on the EEG. The GGE patients with EM who could not be classified into specific GGE syndromes according to ILAE criteria due to the differences of age at seizure onset and/or lack of dominant seizure types were labelled as "GGE-unclassified (GGE-Unc)".

Moreover, 15 age- and gender-matched GGE patients with absence seizures, but without a history of EM, were enrolled as a disease control group. Nine of these patients were diagnosed with childhood absence epilepsy (CAE) and the six remaining patients had juvenile absence epilepsy (JAE).

All patients signed the informed consent forms to participate in the study which was approved by the Local Ethics Committee (number 2012/525-1019).

Laboratory study and data analysis

DNA was isolated from blood samples using a commercial DNA extraction kit according to the manufacturer's instructions. A total of 10 primer pairs were designed for variant screening including all exons and exon-intron boundary sites of the *SLC2A1* gene. Polymerase chain reaction (PCR) was performed in optimized conditions for all the samples. Variant screening was performed via Sanger sequencing for all 10 amplicons.

Results

The mean age at seizure onset and follow-up period of patients (18 females, seven males; mean age: 32.04±8.38) were 10.32±5.36 and 9.6±6.58 years, respectively. Neurological examinations were normal except for two patients with cognitive dysfunction. Fourteen patients (56%) had at least one affected family member diagnosed with epilepsy. In four patients, the age at seizure onset was younger than four years. All the patients were photosensitive except five patients. According to the syndromes, EMA was diagnosed in eight, JAE in four, juvenile myoclonic epilepsy (JME) in two, CAE in two, late-onset absence epilepsy (LOAE) in one, and GGE-Unc in eight patients. At the end of the follow-up period, all patients were on antiepileptic drugs except one patient who was seizure-free without antiepileptic drugs. Clinical and demographic features of the patients are shown in detail in *table 1*.

No *SLC2A1* gene variants were identified in any of the 40 genetically examined patients with GGE. Twenty-one patients were shown to have single nucleotide polymorphisms (SNP) in the EM group. Thirteen individuals showed SNPs clustered in the ninth exon, however, no difference in clinical features, age at seizure onset, family history of epilepsy, or neurological examination was detected in these patients.

Discussion

In our study, the *SLC2A1* gene was investigated in a consecutive homogeneous group of 25 GGE patients exhibiting EM as a possible endophenotypic marker, however, no variant could be detected.

The underlying cause of GGE syndromes is thought to involve mostly a polygenic, complex inheritance pattern, however, we were unable to demonstrate this in our patients despite huge collaborative efforts. The genetic as well as phenotypic heterogeneity of GGE syndromes further complicates the situation probably due to gene expression alterations and low penetrance of the involved genes in different patients. Thus, identification of the major gene defects leading to GGE syndromes does not appear to be straightforward (Poduri and Lowenstein, 2011).

Identification of rare variants requires a detailed analysis of candidate genes. *SLC2A1* was described as one of the most important epilepsy genes according to

Table 1. The clinical and demographic features of the genetic generalized epilepsy patients with eyelid myoclonia.

	Sex	Age (Years)	Follow-up period (Years)	NE	Familial history of epilepsy*	Age at seizure onset	Initial seizure type	Syndrome	EEG Findings	AEDs	Response to therapy
1	F	41	25	CD	∅	6	EM	EMA	GSW/ PS/ ECS/ Fo	LTG	Well
2	F	45	27	N	∅	18	EM, Abs	JAE	GSW/ ECS	LEV/VPA	Poor
3	M	38	13	CD	∅	4	Myo	GGE-Unc	GSW	VPA	Moderate
4	F	47	22	N	∅	15	GTCS	JAE	GSW/ PS	LTG/VPA	Moderate
5	F	38	22	N	∅	5	Myo	GGE-Unc	GSW/ PS	VPA	Well
6	M	37	16	N	Yes (1)	11	EM	GGE-Unc	GSW/ PS	VPA	Well
7	F	26	16	N	Yes (1/FD)	4	EM	EMA	GSW/ PS/ ECS/ Fo	∅	Well
8	F	30	13	N	Yes (1/FD)	10	EM	EMA	GSW/ PS/ ECS	VPA	Well
9	F	48	9	N	Yes (3/FD)	27	GTCS	LOAE	GSW/ PS	VPA	Poor
10	F	31	12	N	∅	12	Myo	JME	GSW/ PS/ ECS	VPA	Moderate
11	F	23	14	N	Yes (2/FD)	4	EM, Myo	EMA	GSW/ PS/ ECS	LTG/VPA	Moderate
12	F	30	6	N	Yes (1/FD)	8	EM	EMA	GSW/ PS/ ECS	VPA	Well
13	M	31	13	N	Yes (1)	10	GTCS	GGE-Unc	GSW/ ECS	VPA	Well
14	M	42	9	N	Yes (1)	2	EM	GGE-Unc	GSW/ PS/ Fo	VPA	Well
15	F	36	7	N	∅	9	EM	CAE	GSW/ PS	VPA	Well
16	F	26	8	N	∅	10	GTCS	EMA+TLE	GSW/ PS/ ECS/ Fo	LEV/VPA/TPM	Moderate
17	F	28	8	N	Yes (3/FD)	15	EM, Abs	JAE	GSW/ PS	LTG	Well
18	F	25	8	N	Yes (2)	9	EM	CAE	GSW/ PS	VPA	Well
19	F	23	8	N	∅	13	EM	JME	GSW/ PS/ Fo	LEV	Moderate
20	M	18	7	N	∅	10	GTCS	EMA	GSW/ PS/ ECS	VPA	Moderate
21	F	25	6	N	∅	6	EM	EMA	GSW/ PS/ ECS	VPA	Well

Table 1. The clinical and demographic features of the genetic generalized epilepsy patients with eyelid myoclonia (*Continued*).

	Sex	Age (Years)	Follow-up period (Years)	NE	Familial history of epilepsy*	Age at seizure onset	Initial seizure type	Syndrome	EEG Findings	AEDs	Response to therapy
22	F	29	3	N	Yes (2)	16	Abs	JAE	GSW/ PS	VPA	Well
23	M	25	6	N	Yes (1/FD)	12	EM	GGE-Unc	GSW/ ECS	VPA	Well
24	F	29	3	N	Yes (1/FD)	11	EM	GGE-Unc	GSW/ ECS/ Fo	VPA	Well
25	M	20	8	N	Yes (1)	11	EM	GGE-Unc	GSW/ PS	VPA/TPM	Moderate

Abs: absence, AED: antiepileptic drugs; CAE: childhood absence epilepsy; CD: cognitive dysfunction; ECS: eye closure sensitivity; EMA: eyelid myoclonia with absences; EM: eyelid myoclonia; F: Female; FD: first-degree relatives; Fo: focal EEG findings; GGE-Unc: genetic generalized epilepsy-unclassified; GSW: generalized spike wave; GTCS: generalized tonic-clonic seizure; JAE: juvenile absence epilepsy; JME: juvenile myoclonic epilepsy; LEV: levetiracetam; LOAE: late-onset absence epilepsy; LTG: lamotrigine; M: male; Myo: myoclonia; N: normal; NE: neurological examination; PS: photosensitivity; TLE: temporal lobe epilepsy; TPM: topiramate; VPA: valproic acid.
*Numbers refer to the number of affected family members.

the ILAE guidelines, mainly because of its association with a treatment option, the ketogenic diet, in drug-resistant patients (Ottman *et al*., 2010). In order to conduct a reasonable search for a candidate gene in a particular group, it is important to select a well-defined homogeneous group (Sander *et al*., 2000; Noebels, 2003). Here, we selected EM as an endophenotypic marker in our study to create a homogeneous GGE subgroup and investigated the *SLC2A1* gene that has previously been found in early-onset absence epilepsies (Arsov *et al*., 2012a, 2012b; Muhle *et al*., 2013).

The phenotypic characteristics of the GLUT1-related spectrum, in terms of epilepsy, have markedly expanded in recent years and *SLC2A1* gene variants have been identified in cases with a diagnosis of "ordinary" GGE with variable age at onset. One study demonstrated *SLC2A1* gene variants in 12% of patients with early-onset absence seizures (Suls *et al*., 2009). Another study also showed a novel non-synonymous *SLC2A1* gene variant in nine affected family members, mainly with absence epilepsies, among 95 patients (Striano *et al*., 2012). However, a study comprising 58 early-onset absence epilepsy patients showed no mutations in the *SLC2A1* gene and no significant differences in terms of responsiveness to mono- or polytherapy (Agostinelli *et al*., 2013). Moreover, Giardano *et al*. also found no mutations in the *SLC2A1* gene in a group of patients with absence seizures starting within the first year of life, suggesting that genetic analysis should be performed only in selected patients (Giordano *et al*., 2013). Given the presence of mildly affected subjects, one can speculate that other modifiers may change the resulting phenotype.

We were unable to find any *SLC2A1* variants that could be responsible for GGE associated with EM. It is also remarkable that no variant was detected in our patients with early seizure onset, however, in our group, we had only four patients with early seizure onset; two were diagnosed with EMA and two were diagnosed with GGE-Unc, thus the overall number was inadequate to draw any conclusions.

Likewise, EM is recognized as a unique generalized seizure type by the ILAE, suggesting a separate pathophysiological origin. On the other hand, EM can be observed in different GGE syndromes, accompanied by other variable phenotypic features, suggesting the existence of a complex inheritance pattern.

A family history of epilepsy was reported in 40-78% of the patients with epilepsy (Poduri and Lowenstein, 2011). In our study, 56% of the patients with GGE-EMA, but only 26.6% of the absence epilepsy group, had at least one family member diagnosed with epilepsy, implying a stronger genetic effect in patients with EM. On the other hand, studies involving twins and relatives of patients with EMA have shown a strong genetic contribution to EMA. (Giannakodimos and

Panayiotopoulos, 1996). It was reported that about 74% of the affected family members of EMA patients show heterogeneous generalized epilepsy phenotypes (Sadleir *et al*., 2012). Based on a recent study investigating *CHD2* mutations in a group of 580 epilepsy patients with photosensitivity and 55 individuals with photoparoxysmal responses without seizure history, *CHD2* gene variants were shown to be associated with the EMA phenotype (Galizia *et al*., 2015). The fact that EM and other GGE syndromes may coexist and share common features is not well-understood from a mechanistic point of view, and the notion that EM may be associated with stronger genetic inheritance still remains.

We observed a clustering of many SNPs (*see supplementary material*) in a particular exon of the *SLC2A1* gene that did not appear to relate to any particular clinical characteristic. The possible role of a modifier effect could not be confirmed at this point.

In conclusion, although the patient number in this study was insufficient to draw any firm conclusions, the absence of *SLC2A1* gene variants indicates that *SLC2A1* does not appear to play a major role in GGE associated with EM. □

Supplementary data.
Supplementary table is available on the www.epilepticdisorders.com website.

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References

Agostinelli S, Traversob M, Accorsic P, *et al*. Early-onset absence epilepsy: *SLC2A1* gene analysis and treatment evolution. *Eur J Neurol* 2013; 20: 856-9.

Appleton RE, Panayiotopoulos CP, Acomb BA, *et al*. Eyelid myoclonia with typical absences: an epilepsy syndrome. *J Neurol Neurosurg Psychiatry* 1993; 56: 1312-6.

Arsov T, Mullen SA, Damiano JA, *et al*. Early onset absence epilepsy: 1 in 10 cases is caused by GLUT1 deficiency. *Epilepsia* 2012a; 53: e204-7.

Arsov T, Mullen SA, Rogers S, *et al*. Glucose transporter 1 deficiency in the idiopathic generalized epilepsies. *Ann Neurol* 2012b; 72: 807-15.

Capovilla G, Striano P, Gambardella A, *et al*. Eyelid fluttering, typical EEG pattern, and impaired intellectual function: a homogeneous epileptic condition among the patients presenting with eyelid myoclonia. *Epilepsia* 2009; 50: 1536-41.

Caraballo RH, Fontana E, Darra F, *et al*. A study of 63 cases with eyelid myoclonia with or without absences: type of seizure or an epileptic syndrome? *Seizure* 2009; 18: 440-5.

Galizia EC, Myers CT, Leu C, *et al*. CHD2 variants are a risk factor for photosensitivity in epilepsy. *Brain* 2015; 138: 1198-207.

Giannakodimos S, Panayiotopoulos CP. Eyelid myoclonia with absences in adults: a clinical and video-EEG study. *Epilepsia* 1996; 37: 36-44.

Giordano L, Vignoli A, Cusmai R, *et al*. Early onset absence epilepsy with onset in the first year of life: a multicenter cohort study. *Epilepsia* 2013; 54: 66-9.

Jeavons PM. Nosological problems of myoclonic epilepsies in childhood and adolescence. *Dev Med Child Neurol* 1977; 19: 3-8.

Leen WG, Klepper J, Verbeek MM, *et al*. Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. *Brain* 2010; 133: 655-70.

Muhle H, Helbig I, Froslev TG, *et al*. The role of *SLC2A1* in early onset and childhood absence epilepsies. *Epilepsy Res* 2013; 105: 229-33.

Noebels JL. Exploring new gene discoveries in idiopathic generalized epilepsy. *Epilepsia* 2003; 44: 16-21.

Ottman R, Hirose S, Jain S, *et al*. Genetic testing in the epilepsies-report of the ILAE Genetics Commission. *Epilepsia* 2010; 51: 655-70.

Poduri A, Lowenstein D. Epilepsy genetics-past, present, and future. *Curr Opin Genet Dev* 2011; 21: 325-32.

Pong AW, Geary BR, Engelstad KM, *et al*. Glucose transporter type I deficiency syndrome: epilepsy phenotypes and outcomes. *Epilepsia* 2012; 53: 1503-10.

Sadleir LG, Vears D, Regan B, *et al*. Family studies of individuals with eyelid myoclonia with absences. *Epilepsia* 2012; 53: 2141-8.

Sander T, Schulz H, Saar K, *et al*. Genome search for susceptibility loci of common idiopathic generalised epilepsies. *Hum Mol Genet* 2000; 9: 1465-72.

Scuderi C, Musumeci SA, Ferri R, *et al*. Eyelid myoclonia with absences in three subjects with mental retardation. *Neurol Sci* 2000; 21: 247-50.

Striano S, Striano P, Nocerino C, *et al*. Eyelid myoclonia with absences: an overlooked epileptic syndrome? *Neurophysiol Clin* 2002; 32: 287-96.

Striano S, Capovilla G, Sofia V, *et al*. Eyelid myoclonia with absences (Jeavons syndrome): a well-defined idiopathic generalized epilepsy syndrome or a spectrum of photosensitive conditions? *Epilepsia* 2009; 50: 15-9.

Striano P, Weber YG, Toliat MR, *et al*. *GLUT1* mutations are a rare cause of familial idiopathic generalized epilepsy. *Neurology* 2012; 78: 557-62.

Suls A, Mullen SA, Weber YG, *et al*. Early-onset absence epilepsy caused by mutations in the glucose transporter *GLUT1*. *Ann Neurol* 2009; 66: 415-9.

Tzadok M, Nissenkorn A, Porper K, *et al*. The many faces of Glut1 deficiency syndrome. *J Child Neurol* 2014; 29: 349-59.