



Epilepsy, cerebral calcifications, and gluten-related disorders: Are anti-transglutaminase 6 antibodies the missing link?

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ABSTRACT

Purpose: Gluten-related disorders (GRDs) are a group of immune-mediated diseases often associated to neurologic manifestations. Epilepsies with cerebral calcifications, with or without coeliac disease (CD), are rare neurological disorders characterized by childhood-onset focal seizures, often refractory to antiepileptic drugs. Transglutaminase 6 antibodies (anti-TG6) have been considered a biomarker for gluten-related ataxia and neuropathy, but their prevalence in epilepsies with cerebral calcifications is unknown. The aim of this study is to evaluate anti-TG6 prevalence in patients with epilepsies and cerebral calcifications.

Method: this was a cross-sectional study conducted at five Italian epilepsy centres. The following groups were included. Group 1: nine patients with CD, posterior cerebral calcifications and epilepsy (CEC); group 2: nine patients with epilepsy and posterior cerebral calcifications, without CD; group 3: twenty patients with focal epilepsy of unknown etiology; group 4: twenty-two healthy controls (HC). All subjects were tested for serological evidence of anti-TG6 IgA and IgG. Differences among groups were analysed using χ^2 test.

Results: anti-TG6 were present in 1/9 subjects (11%) of group 1, 2/9 subjects (22%) of group 2, 0/20 subjects in group 3, 3/22 (13.6%) of HC. No significant difference was found among the 4 groups.

Conclusions: Anti-TG6 do not seem to be associated to epilepsies with cerebral calcifications.

1. Introduction

Gluten-related disorders (GRDs) are a group of immune-mediated diseases with several clinical manifestations triggered by gluten ingestion (1). The three main forms of GRDs are celiac disease (CD), wheat allergy (WA) and non-celiac gluten-sensitivity (NCGS) [1,2]. GRDs often present extraintestinal symptoms. Neurological manifestations have been reported in 10–22% of patients with coeliac disease (CD) and

include gluten ataxia, polyneuropathy, myopathy, epilepsy, leukoencephalopathy, and headache [3]. CD with epilepsy and cerebral calcifications (CEC) is a rare neurological syndrome mainly characterized by childhood-onset focal seizures, often refractory to antiepileptic drugs [4,5]. Posterior cerebral calcifications may also be found in asymptomatic coeliac patients as well as in patients without CD [4,5]. Calcifications have been initially assumed to be secondary to vitamin deficiencies due to malabsorption (e.g. folate, vitamins B12, D, and E)

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Table 1
Main clinical data of group 1.

Patients	Age (y)	Age at seizure onset (y)	Anti-epileptic treatment	Calcification localization	CD diagnosis	Gluten-free diet	Anti-TG6 positivity
1	49	2	CBZ, PB	occipital	Histological + Serological	yes	no
2	41	17	OXC, CLB	occipital	Serological	yes	no
3	40	8	VPA, LTG, RUF	parieto-occipital	Serological	yes	no
4	41	6	CBZ, CLB	Parieto-occipital	Serological	yes	no
5	42	5	FLB, CLB, VPA, VNS	occipital	Histological + serological	yes	no
6	45	19	OXC	temporo-occipital	Histological + serological	yes	no
7	53	18	TPM, PER, CBZ	occipital	Serological	yes	no
8	44	1	OXC, LTG, PGB, CLB, PER	occipital	Histological + serological	yes	no
9	46	9	FLB, CLB	parietal	Histological + serological	yes	yes

CBZ: Carbamazepine, CLB: Clobazam, FLB: felbamate; LEV: Levetiracetam, LTG: Lamotrigine, OXC: Oxcarbazepine, PB: Phenobarbital, PGB: Pregabalin, PHT: Phenytoin, RUF: Rufinamide, TPM: Topiramate, VNS: Vagus Nerve Stimulation, VPA: Valproic acid.

[4,5]. However, as neurological manifestations can arise without enteropathy, immune-mediated mechanisms have been postulated [5].

Transglutaminases (TG) are calcium-dependent enzymes, found in the vasculature, gut, and brain [6]. They catalyze post-translational modification of glutamine residues through isopeptide linkage, deamidation, or esterification. Different TG isoenzyme antibodies have been linked to specific extra-intestinal syndromes, the best-characterized being TG3 antibodies in dermatitis herpetiformis [6]. Antibodies against TG6 (anti-TG6), a recently identified class [7], have been thought to be a more specific marker for neurological manifestations, with the median TG6 antibody concentration being significantly higher than anti-TG2 in patients with CD-associated neurological complications [7,8]. In a post-mortem analysis of patients with gluten ataxia, TG6 were widely distributed in the brain [7].

Elevated levels of anti-TG6 have also been found in the serum of one patient with CEC, suggesting a role of these Ab in the pathogenesis of epilepsies with brain calcifications [9].

The aim of our study is to investigate the prevalence of anti-TG6 in patients with epilepsies with cerebral calcifications.

2. Subjects and methods

The Ethical Committee of the Great Metropolitan Hospital Bianchi-Melacrino-Morelli, Reggio Calabria, Italy approved the study. All patients provided written informed consent.

2.1. Patients selection

This was a cross-sectional study conducted at five Italian epilepsy centres (Regional Epilepsy Centre, Great Metropolitan Hospital “Bianchi-Melacrino-Morelli”, Reggio Calabria; Child Neurology Unit, Bellaria Hospital, IRCCS – Institute of Neurological Sciences, Bologna, Italy; Department G.F. Ingrassia, Section of Neurosciences, University of Catania; Epilepsy Centre, Department of Neurological Sciences and Mental Health, “La Sapienza” University of Rome; Epilepsy Centre, Department of Neuroscience, Odontostomatological and Reproductive Sciences, University of Naples Federico II, Naples). The following groups of subjects were included. Group 1: patients with CD, posterior cerebral calcifications and epilepsy (CEC), with or without gluten-free diet; Group 2: patients with epilepsy and posterior cerebral calcifications, without CD; Group 3: patients with focal epilepsy of unknown etiology; Group 4: healthy controls (HC) (without signs or symptoms of enteropathy or any neurological diseases) attending the Regional Epilepsy Centre of Reggio Calabria, Italy.

The diagnosis of CD in patients from group 1 and 2 was made according to the new European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Celiac Disease (ESPGHAN). All subjects from group 1 and 2 underwent determination of TG2 IgA and IgG Ab, endomysial IgA and IgG Ab (EMA), gliadine IgG and IgA Ab (AGA) titer, as well as HLA test for DQ2/DQ8.

The decision to proceed with a duodenal biopsy was taken according to ESPGHAN recommendations. The epilepsy type was classified according to the 2017 ILAE criteria [10]. Posterior cerebral calcifications were detected by mean of CT scan and brain MRI.

Subjects from group 3 and 4 who were found positive for TG6-Ab were tested for AGA, EMA, TG2-Ab and HLA DQ2 and DQ8.

2.2. Serum testing

All subjects were tested for serological evidence of IgA and IgG anti-TG6. Serological testing was performed using ELISA (ZediXclusive anti-TG6 IgA and IgG ELISA kits).

Titer cut-off for TG6 IgA was 41 U/ml (TG6-Abtype IgA > 41 U/ml positive; TG6-Ab type IgA < 26 U/ml negative; TG6-Ab type IgA in the range 26–41 U/ml borderline). Titer cut-off for TG6 IgG was 44 U/ml (TG6 –Ab IgG > 44 U/ml positive; TG6 –Ab IgA < 28 U/ml negative; TG6–Ab IgA in the range 28–44 U/ml borderline).

2.3. Statistical analysis

Planned comparisons included the positivity and negativity to IgG and IgA TG6. The frequencies of these dichotomous variables were compared using χ^2 test.

3. Results

Sixty patients were ultimately recruited (group 1: 9 patients; group 2: 9 patients; group 3: 20 patients; group 4: 22 patients).

3.1. Group 1 (patients with CEC)

One of these 9 patients (11%) (#9) was positive for TG6 IgG (titer 45.5 U/ml). Clinical data of patients belonging to group 1 are summarized in Table 1. Eight/9 patients had focal onset impaired awareness seizures, 1 patient (#8) had focal onset aware seizures.

3.2. Group 2: patients with epilepsy and posterior cerebral calcifications, without CD

Two of these 9 patients (22%) (#6,9) were positive for anti-TG6. In particular, patient 6 was positive for IgA (titer 155,09 U/ml) with borderline IgG value (38,57 U/ml), while patient 9 had a borderline anti-TG6 IgA titer (29,06 U/ml). Clinical data of patients belonging to group 2 are summarized in Table 2. Seven/9 patients had focal onset impaired awareness seizures, 2 patients (#2,7) had focal onset aware seizures. Despite the absence of a diagnosis of GRDs, patient #6 was on gluten-free diet.

Table 2
Main clinical data of group 2.

Patients	Age (y)	Age at seizure onset (y)	Anti-epileptic treatment	Calcification localization	Anti-TG6 positivity
1	31	21	TPM	Occipital	no
2	41	20	OXC	parieto-occipital dx	no
3	46	6	TPM, LEV	occipital	no
4	35	25	LEV	occipital	no
5	46	1	LTG, CLB, CLN, CBZ, PER, VNS	occipital	no
6	19	16	LTG	occipital	yes
7	41	12	None	temporo-parieto-occipital	no
8	36	2	CLB, CBZ, TPM, LEV, PB	parieto-occipital	no
9	51	8	CBZ, VPA, TPM	occipital	yes

CBZ: Carbamazepine, CLB: Clobazam, CLN: Clonazepam, LEV: Levetiracetam, LTG: Lamotrigine, OXC: Oxcarbazepine, PB: Phenobarbital, PER: Perampanel, TPM: Topiramate, VNS: Vagus nerve stimulation, VPA: Valproic acid.

3.3. Group 3: patients with focal epilepsies of unknown etiology

Seventeen/20 patients had focal onset impaired awareness seizures, 3 patients had focal onset aware seizures. None of these 20 patients was positive for anti-TG6.

3.4. Group 4: healthy controls

Three of 22 HC (13,6%) were positive for IgG only (48,75 U/ml, 61.8 U/ml and 107 U/ml respectively). These 3 subjects were negative for serological screening of CD but 1/3 was positive for HLA DQ2.

3.5. Comparison among groups

The prevalence of Ab-TG6 among groups was not significantly different (p value = 0.1521 for IgA; p value = 0.2860 for IgG).

4. Discussion

In the present study, we showed that anti-TG6 are equally common in patients with epilepsies associated with cerebral calcifications, with or without CD, in patients with epilepsies of unknown origin and in healthy controls. In particular, in patients with epilepsy and cerebral calcifications without CD, only 2 patients (22%) were positive for anti-TG6; of note, one of these 2 patients was on gluten-free diet despite the lack of diagnosis of GRDs.

Zis et al. found higher prevalence of anti-TG6 in patients with gluten neuropathy (50%) as compared to healthy controls (4%) [11]. Hadjivassiliou et al. showed higher prevalence anti-TG6 in patients with gluten ataxia, as compared to other form of ataxia (32% of patients with idiopathic sporadic ataxia, 73% of patients with gluten-ataxia, 32% of subjects with CD, 5% of subjects with other neurodegenerative disease, 4% of healthy controls) [8]. The authors concluded that anti-TG6 are a sensitive and specific marker of gluten-ataxia [8].

According to our results, anti-TG6 cannot be considered a diagnostic biomarker for epilepsy associated to cerebral calcifications, with or without CD, and the pathogenesis of these conditions remains to be elucidated.

This study has some limitations. First, all patients belonging to group 1 were on gluten-free diet and this may reduce anti-TG6 titers [8]. However, it was unfeasible to withdraw gluten-free diet in those patients. Another limit of this study is represented by the small sample size, in particular of group 2, which may have led to a false negative result. This is largely caused by the rarity of epilepsy associated to cerebral calcifications without CD [4,5]. Anti-TG6 have never been searched in subjects with epilepsy and cerebral calcifications, without CD. Moreover, anti-TG6 have been searched in one patient only with

CEC [9]. Due to the positivity of anti-TG6 in this patient, authors suggested a role of anti-TG6 in the pathogenesis of epilepsies with brain calcifications [9]. Our findings, despite the intrinsic limitation due to the case-control study design, argue against this hypothesis and prompt further research in this field. Notably, transglutaminases are found both in gut and brain. The relationship between gut microbial system, intestinal inflammation and brain disorders has been extensively studied in recent years, both in animal models and in humans [12,13]. Thus, intestinal inflammation should be evaluated in epilepsy patients with and without cerebral calcifications.

In conclusion, this is the first study evaluating anti-TG6 prevalence in patients with epilepsy with cerebral calcifications. Anti-TG6 do not seem to be associated with epilepsies with cerebral calcifications.

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Declaration of Competing Interest

None.

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