Epilepsy Research (2013) **105**, 86—91

journal homepage: www.elsevier.com/locate/epilepsyres

Newly-diagnosed pediatric epilepsy is associated with elevated autoantibodies to glutamic acid decarboxylase but not cardiolipin

Kadi Veri[a,1], **Oivi Uibo**[b,1], **Tiina Talvik**[b,1], **Inga Talvik**[b,1], **Kaja Metsküla**[c,2], **Aita Napa**[d,1], **Ulvi Vaher**[d,1], **Eve Õiglane-Šlik**[b,1], **Reet Rein**[d,1], **Anneli Kolk**[b,1], **Aili Traat**[d,1], **Raivo Uibo**[c,*]

[a] *Department of Pediatrics, University of Tartu, Lunini 6, Tartu 51014, Estonia*
[b] *Department of Pediatrics, University of Tartu, Children's Clinic of Tartu University Hospital, Lunini 6, Tartu 51014, Estonia*
[c] *Immunology Group, Institute of Biomedicine and Centre for Translational Medicine, University of Tartu, Ravila 19, Tartu 50411, Estonia*
[d] *Children's Clinic of Tartu University Hospital, Lunini 6, Tartu 51014, Estonia*

Received 26 July 2012; received in revised form 4 February 2013; accepted 12 February 2013
Available online 25 March 2013

KEYWORDS
Epilepsy;
Autoantibodies;
Glutamic acid decarboxylase;
Anti-cardiolipin autoantibodies;
Children

Summary Glutamic acid decarboxylase autoantibodies (GADA) and anti-cardiolipin autoantibodies (ACA) have been detected in adult subjects with epilepsy, though the functional implications of these findings are a matter of debate. This study aimed to determine the prevalence of GADA and ACA and to investigate their clinical significance in pediatric subjects with newly-diagnosed epilepsy. For this purpose GADA and ACA were assessed by enzyme-linked immunosorbent assays in 208 pediatric patients with newly-diagnosed epilepsy and 128 controls. The clinical data (results of electroencephalography, magnetic resonance imaging, 6-month outcome etc.) was compared to antibody test results. Our study revealed GADA in 14 (6.7%) patients with epilepsy and in 1 (0.8%) control, which was a statistically significant difference ($P = 0.010$; Chi-square test). The GADA-positive and -negative patients had similar clinical characteristics. The prevalence of ACA in patients with epilepsy (6.3%) was not significantly different than controls (2.6%). These results suggest that GADA is associated with epilepsy in a subgroup of newly-diagnosed pediatric patients. Further studies are required to determine the prognostic significance and pathogenic role of GADA among pediatric subjects with epilepsy.

* Corresponding author. Fax: +372 7374232.
E-mail addresses: kadi.veri@kliinikum.ee (K. Veri), oivi.uibo@kliinikum.ee (O. Uibo), tiina.talvik@kliinikum.ee (T. Talvik), inga.talvik@kliinikum.ee (I. Talvik), kaja.metskyla@kliinikum.ee (K. Metsküla), aita.napa@kliinikum.ee (A. Napa), ulvi.vaher@kliinikum.ee (U. Vaher), eve.oiglane-slik@kliinikum.ee (E. Õiglane-Šlik), reet.rein@kliinikum.ee (R. Rein), anneli.kolk@kliinikum.ee (A. Kolk), aili.traat@kliinikum.ee (A. Traat), raivo.uibo@ut.ee (R. Uibo).
[1] Fax: +372 7319608.
[2] Fax: +372 7374232.

0920-1211/$ — see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.eplepsyres.2013.02.009

Introduction

Recent studies suggest an increased prevalence of various autoantibodies in subjects with epilepsy (Irani et al., 2011). Specifically, glutamic acid decarboxylase autoantibodies (GADA), anti-cardiolipin autoantibodies (ACA) or autoantibodies to other phospholipids, glutamate receptors, and voltage-gated potassium channels have been reported to be associated with epilepsy. Among adult subjects with epilepsy, GADA and ACA have been shown to be the most common and have therefore attracted considerable attention for immunological characterization of the disease (Liimatainen et al., 2010; Falip et al., 2012).

Antibodies to glutamic acid decarboxylase (GAD), the rate-limiting enzyme of gamma-aminobutyric acid (GABA) synthesis, have been largely studied in type 1 diabetes mellitus (Baekkeskov et al., 1990) and in several neurological conditions, including stiff-person syndrome (Solimena et al., 1990; Vianello et al., 2005), cerebellar ataxia (Vianello et al., 2003, 2005), palatal myoclonus (Nemni et al., 1994; Vianello et al., 2005) and limbic encephalitis (Matà et al., 2008). There are some reports of increased GADA levels in idiopathic generalized epilepsies (Aykutlu et al., 2005; Striano et al., 2008), but GAD autoimmunity has mostly been associated with localization-related epilepsies, in particular with temporal lobe epilepsy of unknown etiology (Giometto et al., 1998; Errichiello et al., 2009; Liimatainen et al., 2010) and drug-resistant epilepsy (Peltola et al., 2000; McKnight et al., 2005; Yoshimoto et al., 2005; Vulliemoz et al., 2007). The clinical significance of elevated GADA levels in subjects with epilepsy is still widely debated (Bien and Scheffer, 2011).

Although ACA are a common marker for rheumatic diseases, they have also been detected in subjects with epilepsy (Verrotti et al., 2003; McKnight et al., 2005). Increased prevalence of ACA in subjects with partial epilepsy has been associated with a long duration of disease and poor seizure control (Ranua et al., 2004).

The aim of the current study was to determine the prevalence of GADA and ACA, and to elucidate their clinical significance among pediatric subjects with newly-diagnosed epilepsy.

Methods

This prospective study comprised 208 pediatric patients (mean age of 7.8 years; range from 1 month to 19 years), including 109 males, who were admitted to the Children's Clinic of Tartu University Hospital, one of two tertiary pediatric care centers of Estonia, between January of 2009 and April of 2011. All these patients had newly-diagnosed epilepsy, which was classified by a neurologist and a neurophysiologist according to the recommendations of the International League Against Epilepsy (ILAE, 1989) and confirmed. Neonatal seizures and cases with only febrile seizures were excluded. All subjects underwent an awake and sleep-deprived video-electroencephalography (EEG), and brain *magnetic resonance* imaging (MRI). The effectiveness of antiepileptic treatment was evaluated 6 months later. Clinical characteristics of the study subjects are presented in Table 1.

Table 1 Clinical data from 208 children with epilepsy.

General features	No. (%)
Sex	
Males	109 (52.4)
Females	99 (47.6)
Mean age (years)	7.8
Coexisting diabetes	1 (0.5)
Epilepsy type	
Idiopathic	113 (54.3)
Focal	69 (33.2)
Generalized	44 (21.1)
Cryptogenic	25 (12.0)
Symptomatic	34 (16.3)
Focal	32 (15.4)
Generalized	2 (0.9)
Unclassified	36 (17.3)

The control group [$n = 128$ (64 males), mean age 9.5 years; range from 2 to 18 years] included subjects with functional urinary (enuresis) and gastrointestinal (abdominal pain, constipation) disorders admitted to the Children's Clinic of Tartu University Hospital. A complete blood count and C-reactive protein levels were used to exclude acute illness. Clinical records were reviewed to exclude coexisting autoimmune and neurological disorders.

In all children venous blood samples for autoantibody analyses were obtained. Serum was divided into aliquots and stored at −20 °C. GADA (GAD65 isoform) were measured using enzyme-linked immunosorbent assay (ELISA, RSR Ltd., UK) where GAD isoform of 65 kD served as antigen. Values ≥5 U/ml were considered positive for GADA presence. According to the 2009 Diabetes Autoantibody Standardization Program (DASP), this method has high sensitivity and specificity for GADA detection. ACA were detected by ELISA (Euroimmun AG, Germany) with values ≥12 RU/ml considered positive. This assay detects IgM, IgG and IgA antibodies against cardiolipin and was performed under UKNEQAS quality assurance control. In patients with epilepsy blood was taken before the antiepileptic treatment.

Written informed consent was obtained from all studied subjects and/or their parents, and the study was approved by the Research Ethics Committee of the University of Tartu.

Statistical analysis was performed using Chi-square test and Fisher's exact test.

Results

GADA were detected in 14 (6.7%) patients with epilepsy and in 1 (0.8%) control subject with statistically significant difference (Chi-square test $P = 0.010$). The GADA-positive control subject (antibody level of 6 U/ml) was a 12-year-old male with enuresis but no other signs of disease. Of the 14 GADA-positive patients, 5 had focal idiopathic epilepsy, 2 had focal symptomatic epilepsy, 2 had generalized idiopathic epilepsy, 1 had generalized symptomatic epilepsy and 4 had unclassified epilepsy. The GADA-positive and -negative subjects showed similar clinical characteristics.

Most patients with epilepsy ($n = 11$) displayed a low GADA level (5—38 U/ml), but three had GADA values >50 U/ml,