Seizure 20 (2011) 438–441

Contents lists available at ScienceDirect

Seizure

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

Cardiolipin and β_2-Glycoprotein I antibodies associate with cognitive impairment and seizure frequency in developmental disorders

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ARTICLE INFO

Article history:
Received 29 November 2010
Received in revised form 22 January 2011
Accepted 31 January 2011

Keywords:
Epilepsy
Phospholipid antibodies
Intellectual impairment
Seizure
Developmental disorder

ABSTRACT

Cardiolipin (CL) and β_2-Glycoprotein I (β_2-GpI) antibodies have been shown to associate with various neurological symptoms including seizures and cognitive dysfunction. Here we studied the prevalence of CL, β_2-GpI and antinuclear (ANA) antibodies in 74 patients with various developmental disorders with epilepsy and 70 healthy controls. Developmental disorders were classified into genetic syndromes and diseases, genetic and/or acquired conditions, cortical dysgenesias and acquired encephalopathias. IgM-CL and β_2-GpI antibodies were significantly more common in patients (46% vs. 20%, $p < 0.001$ and 10% vs. 0%, $p < 0.05$). Patients with most frequent seizures were more likely to have IgM-CL antibodies. The risk for positive IgM-CL, IgG-CL and β_2-GpI antibodies increased concomitantly with increasing intellectual disability. Present data demonstrates that epilepsy with frequently recurring seizures may be associated with secondary immune system activation.

1. Introduction

Phospholipid (PL) antibodies are a heterogeneous group of autoantibodies binding to phospholipid-like antigens like cardiolipin (CL) and phosphatidylserine (PS). β_2-Glycoprotein I (β_2-GpI) is a plasma protein binding to negatively charged macromolecules and structures, such as phospholipids, DNA, platelets and mitochodria.[1] β_2-GpI mRNA is also expressed in neurons, astrocytes and in brain endothelial cells.[2] Phospholipid and β_2-GpI antibodies have reported to bind to neuronal tissue.[3–6]

High systemic titres of these antibodies are characteristic to primary antiphospholipid syndrome (APS), with systemic manifestations of recurrent spontaneous pregnancy loss, thrombosis, thrombocytopenia, as well as various central nervous system (CNS) symptoms.[7] These neurological symptoms include chorea, psychosis, depression, cognitive dysfunction and dementia.[7,8] Seizures are relatively well characterized neurological symptoms associated with PL antibodies, especially in patients with systemic lupus erythematosus (SLE).[9,10] However, PL antibodies have been found in patients with epilepsy without SLE.[11–13]

In the present study we aimed to study the role of CL, β_2-GpI and antinuclear (ANA) autoantibodies in different types of developmental disorders. We have especially focused on whether these antibodies are associated with the level of cognitive dysfunction, etiological factors underlying intellectual disability and epilepsy, specific epilepsy syndromes or frequency of seizures.

2. Materials and methods

The inclusion criteria for the study were a developmental disorder due to a genetic or structural cause with at least five-year history of refractory seizures despite of adequate treatment. Of 77 consecutively recruited patients, written informed consent was obtained from guardians of 74 patients. A total of 70 healthy blood donors were used as control subjects.

The patients' blood samples were taken in connection to routine laboratory tests. We used commercially available assays to determine CL antibody titres (Quanta Lite ACA IgG and IgM; B_2 GPI, IgG; INOVA Diagnostics, San Diego, CA, USA). Patients and controls with positive results in IgG-CL assay were also tested for distinct IgG class β_2-GpI antibodies. Antinuclear (ANA) antibodies were measured indirectly using Hep-2 cells as antigens (INOVA Diagnostics, San Diego, CA, USA), and presence of antibodies were determined using fluoromicroscopy. The limit values between positive and negative results were 12.5 international MPL units for IgM-CL antibodies; 15 international GPL units for IgG-CL antibodies, and 20 international SGU units for β_2-GpI antibodies.

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doi:10.1016/j.seizure.2011.01.018

Table 1
Etiological groups and specific diagnoses of the 74 study patients.

Etiological groups	Diagnosis
Genetic syndromes (15)	Down syndrome (4), X-linked mental retardation (3), trisomy 15q (2), Angelman (1), deletion 6q (1), autosomal dominant familial disorder (1), Kabuki (1), unidentified dysmorphic syndrome (2)
Genetic diseases (7)	Aspartylglucosaminuria (2), spinocerebellar ataxia (1), neurofibromatosis type 2 (1), MELAS (1), x-linked familial (1), Dravet (1)
Genetic/acquired condition (6)	Infantile autism (4), unspecific intellectual disability with generalized epilepsy (2)
Cortical dysgenesias (15)	Polymicrogyria (6), temporal dysmyelination (4), microcephalia (2), schizenkefalia (1), pachygyria (1), lissenencephalia (1)
Acquired encephalopathias (31)	post-asphyxial (11), post-infectious (8), mesial temporal sclerosis (5), early severe epilepsy (4) and post-traumatic (3)

Pearson Chi-square test, Fisher's exact test and logistic regression analysis served in statistical analysis. A p value of 0.05 or less was considered statistically significant. The ethical committee of Tampere University Hospital approved the study design.

3. Results

3.1. Clinical data

The study group comprised of 74 patients (31 females and 43 males, age range 15–61, mean age 35 years) and 70 controls (age range 16–65 years, mean age 36 years). All patients had undergone a detailed analysis of their condition including video-EEG, brain MRI as well as consultation of a clinical geneticist and psychologist. The patients' etiological diagnoses in detail (classified into genetic syndromes, genetic diseases, genetic and/or acquired conditions, cortical dysgenesias and acquired disorders) are presented in Table 1 and epilepsy diagnoses in Table 2. Sixty-four patients (86%) experienced first seizures during childhood or adolescence. Twelve patients showed hippocampal sclerosis (eight had an acquired encephalopathia and four a genetic disorder) in brain MRI. Two patients acted at subnormal intelligence, 7 at the level of mild (IQ 69–50), 4 at moderate (IQ 49–35), 30 at severe (IQ 34–20), and 31 at the level of profound (IQ < 20) intellectual disability (ID). Nine patients suffered from 1 to 11 seizures yearly, 27 from 1 to 3 seizures monthly, 21 from 1 to 6 seizures weekly, and 17 patients from daily seizures.

All patients except one were on polytherapy. Fifty-five patients received valproate, 28 topiramate, 25 lamotrigine, 16 oxcarbatsepine, 10 levetirasetame, 9 carbamazepine, 9 clobatsame, 8 clonazepam, 7 vigabatrine, 6 gabapentine, 5 phenytoin, 2 ethosuximide, 2 azetatsolamide, and 2 phenobarbitale.

3.2. Antibody tests

3.2.1. Patients and controls

Positive titres of IgM-CL antibodies were more frequent in patients (46%) than in controls (20%) ($p < 0.001$; Fisher's exact test, Table 2). The prevalence of positive IgG-CL antibodies did not differ between patients (16%) and controls (13%), however, 10% of patients had β_2-GpI antibodies while no β_2-GpI antibodies were detected in controls ($p < 0.05$; Fisher's exact test, Table 2). The prevalence of ANA antibodies was the same among patients and controls. The patient's age, gender, onset age of epilepsy or medication did not relate to the prevalence of PL or ANA antibodies.

3.2.2. Etiology of developmental disorder

The prevalence of positive antibody titres in different etiological groups is presented in Table 2. Autoantibodies were not specific to any of the etiological groups, but were distributed within all groups studied. The prevalence of positive antibodies was highly dependent of the number of patients in each group (Table 2).

3.2.3. Epilepsy syndrome and seizure frequency

Autoantibodies studied were not specific to any of the epileptic syndromes. There were no statistically significant differences between epileptic syndromes, although IgM-CL antibodies were most frequent in Lennox-Gastaut syndrome (Table 2). Prevalence of antibodies was highly dependent of the group size. We found no association between positive antibodies and presence of hippo-

Table 2
Prevalence of positive antibodies in controls and patients according to etiology and epilepsy syndrome.

	IgM-CL (%)	IgG-CL (%)	β_2-GpI (%)	ANA (%)
Controls ($n = 70$)	14 (20)	9 (13)	0	8 (11)
All patients ($n = 74$)	34 (46)***	12 (16)	7 (10)*	8 (11)
Etiology				
Genetic syndrome ($n = 15$)	5 (33)	4 (27)	1 (7)	0
Genetic disease ($n = 7$)	3 (43)	1 (14)	1 (14)	0
Genetic/acquired condition ($n = 6$)	2 (33)	0	0	1 (17)
Cortical dysgenesias ($n = 15$)	7 (47)	1 (7)	1 (7)	1 (7)
Acquired encephalopathia ($n = 31$)	17 (55)	6 (19)	4 (13)	6 (19)
Epilepsy syndrome				
Lennox-Gastaut syndrome ($n = 18$)	12 (67)	4 (22)	2 (11)	4 (22)
CSWSS ($n = 4$)	0	0	0	0
Generalized epilepsy (degenerative) ($n = 6$)	2 (33)	2 (33)	0	0
Generalized epilepsy (idiopathic) ($n = 5$)	0	0	0	0
TLE with HS ($n = 12$)	5 (42)	2 (17)	1 (8)	2 (17)
Extra-TLE focal/multifocal epilepsy ($n = 26$)	12 (44)	4 (15)	4 (15)	2 (7)
Reflex epilepsy ($n = 1$)	1	0	0	0
Infantile spasms ($n = 1$)	1	0	0	0
Dravet syndrome ($n = 1$)	1	0	0	0

Abbreviations: Ab, antibody; ANA, antinuclear antibody; CL, cardiolipin; CSWSS, continuous spike-waves during slow-wave sleep epilepsy syndrome; Gp, glycoprotein; HS, hippocampal sclerosis; IgG, immunoglobulin class G; IgM, immunoglobulin class M; TLE, temporal lobe epilepsy.
Statistical difference between patients and controls: *** ($p < 0{,}001$), and * ($p < 0{,}05$; Fisher's exact).