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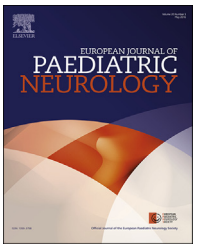

Original article

Autoantibodies to neuronal antigens in children with focal epilepsy and no *prima facie* signs of encephalitis

Peter Borusiak [a,*], *Ulrich Bettendorf* [b], *Gert Wiegand* [c], *Thomas Bast* [d], *Gerhard Kluger* [e,f], *Heike Philippi* [g], *Dieter Münstermann* [h], *Christian G. Bien* [i]

[a] *Center for Child and Adolescent Medicine, HELIOS Hospital Wuppertal, Germany*
[b] *KiPraHi – Neuropediatric Medical Office Hirschaid, Germany*
[c] *Department of Neuropediatrics, University Medical Center Schleswig-Holstein (UKSH, Campus Kiel), Kiel, Germany*
[d] *Epilepsy Center Kork, Kehl, Germany*
[e] *Epilepsy Center Vogtareuth, Germany*
[f] *Paracelsus Medizinische Privatuniversität (PMU) Salzburg, Austria*
[g] *Center of Developmental Neurology, Frankfurt, Germany*
[h] *Labor Krone, Bad Salzuflen, Germany*
[i] *Epilepsy Center Bethel, Krankenhaus Mara, Bielefeld, Germany*

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ABSTRACT

Objective: There is increasing awareness of neuronal autoantibodies and their impact on the pathogenesis of epilepsy. We investigated children with focal epilepsy in order to provide an estimate of autoantibody frequency within a pediatric population without *prima facie* evidence of encephalitis using a broad panel of autoantibodies. This was done to assess the specificity of antibodies and to see whether antibodies might be of modifying influence on the course of focal epilepsies.

Method: We searched for autoantibodies in 124 patients with focal epilepsy (1–18 years; mean 10; 6 years). Sera were tested using a broad panel of surface and intracellular antigens.

Results: We found autoantibodies in 5/124 patients (4%): high-positive GAD65 antibodies ($n = 1$), low-positive GAD65 antibodies ($N = 1$), VGKC complex antibodies not reactive with LGI1 or CASPR2 ($n = 3$). We did not find any distinctive features distinguishing antibody positive patients from those without antibodies.

Conclusions: The antibodies found in this cohort are probably neither disease-specific nor pathogenic. This has been suggested before for these antigenic targets. Moreover, they do not seem to modify disease severity in the antibody-positive epilepsy patients.

* *Corresponding author*. Center for Child and Adolescent Medicine, HELIOS Hospital Wuppertal, Witten/Herdecke-University, Heusnerstr. 40, D-42283 Wuppertal, Germany. Tel.: +49 202 8963850; fax: +49 202 8963848.
E-mail address: peter.borusiak@helios-kliniken.de (P. Borusiak).

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1. Introduction

There is an increasing interest in immunopathogenic mechanisms in the epilepsies. During the last decade, specific autoantibodies in patients with autoimmune encephalitides have been identified. About 80% of these patients with pathogenic effects of the adaptive immune system have seizures.[1] "Autoimmune epilepsies", i.e., autoimmune encephalitides with seizures as the only or the predominant symptom, have been diagnosed not only in adults but also in pediatric patients. In series selected by the clinical suspicion of autoimmune encephalitis, up to 44% of children had antineural antibodies,[2] One may hypothesize that not only patients with typical encephalitic features but also other epilepsy cases may have an autoantibody-related etiology. Recent data linking autoimmunity in general to a higher risk of epilepsy may point into this direction: Ong et al. demonstrated a significantly heightened risk of epilepsy in patients with other autoimmune diseases with an odds ratio of 3.8 in a large population based study with more than 2.5 million patients. In children, this odds ratio was even as high as 5.2.[3] Studies in distinct autoimmune conditions point to the same direction: The prevalence of epilepsy is eight times higher in patients with systemic lupus erythematosus (SLE) than in the general population, multiple sclerosis and epilepsy may occur together more commonly than by chance.[4,5]

The specificity of antibodies has been estimated by the plausible association with specific encephalitic syndromes. Here, those clinical constellations were deliberately excluded. So, first of all, this cohort is a kind of "negative control" cohort for antibody specificity. That means: any antibodies found in this cohort may be considered as being of doubtful specificity.

In addition, we considered the possibility that antibodies in this cohort even though not being qualitatively specific (syndrome-specific), they might still be related "quantitatively" to the severity of the epilepsies, i.e., might be associated with a more severe, pharmacoresistant phenotype. Children with structural-metabolic epilepsies more often have persisting seizures despite adequately chosen drugs than e.g. patients with typical benign epilepsy with centrotemporal spikes (BECTS). Yet again, some patients with presumed problematic constellation will become seizure free without any problems whereas others without any obvious findings might run a devastating course. A comparison of patients with well controlled epilepsies with a cohort of difficult to treat patients might add information on a possible modifying role of autoimmune mechanisms on the course of epilepsies in children.

2. Methods

2.1. Participants

The study population consisted of patients ≥1 and ≤18 years with focal epilepsy. Two different groups were recruited depending on the course of epilepsy of last six months irrespective of autoantibodies which were analyzed *en bloc* at the end of the study. The patients were classified before the antibody analysis was done in terms of epilepsy type and treatability. We did not intend to include all patients with epilepsy at the participating centers but rather to create two distinctive groups: well controlled epilepsies compared to a cohort of difficult to treat epilepsies. In order to avoid any overlap the first group consisted of patients without severe problems concerning seizure control ("easy to treat group" – ett, group 1). Inclusion criteria were a maximum of one seizure during the last six months, a present combination therapy of at most two drugs and not more than three different drugs for long term treatment in their treatment history. Additional emergency treatment with diazepam, lorazepam, etc. in the past was accepted. The other group consisted of patients with difficult to treat epilepsies. Criteria were persisting seizures – at least two seizures during the last six months despite adequately chosen drugs – and treatment with at least three different drugs in the past ("difficult to treat group" – dtt, group 2). Patients not completely fulfilling the criteria of respective groups were not included in the study. This also accounted for children in whom either the patients themselves or their parents were not willing to participate.

In Germany pediatric patients with an uncomplicated course are mainly treated in specialized medical practices. Depending on regional needs also some ambulatory services of hospitals are allowed to treat patients on an outpatient base. For patients with a more complicated course epilepsy centers serve as tertiary referral centers with special emphasis on difficult to treat epilepsies. As we intended to include patients from both ends of the spectrum, different sites were asked to participate. No financial compensation was paid for the inclusion of a patient and the documentation for study purpose and work-up. Thus, we anticipated that due to workload most centers might be able to recruit just a limited series of patients. To keep a possible bias as small as possible we tried to include patients at least as a consecutive series in different centers.: Center for Child and Adolescent Medicine, HELIOS Hospital Wuppertal, Epilepsy Center Kork, Epilepsy Center Vogtareuth, Neuropediatric Department of the University Kiel, the Neuropediatric Medical Office Hirschaid and the Center of Developmental Neurology Frankfurt included the patients and collected blood samples. Patients' history including detailed information on the course of the epilepsy was derived from patients' records and completed by recent history given either by the patients themselves or their parents. Seizures and epilepsies were classified according to the new organization of the epilepsies by the participating centers as provided by the International League against Epilepsy (ILAE).[6]

2.2. Laboratory methods

After obtaining informed consent of patients and their parents the additional blood samples for study purpose were taken on the different study sites when a routine blood test was ordered. Cerebrospinal fluid testing was not part of the study. Blood samples were centrifuged at 5.000 U/min and serum was stored at study sites at a temperature of −20 °C or

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