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Anti-Purkinje cell antibody as a biological marker in attention deficit/hyperactivity disorder: A pilot study

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ABSTRACT

An autoimmune hypothesis has been suggested for several disorders in childhood. The aim of the study was to clarify the role of the cerebellum in ADHD and to evaluate the possible association between anti-Yo antibodies and ADHD. The presence/absence of antibodies was tested by indirect immunofluorescence assay on 30 combined subtype ADHD children, on 19 children with other psychiatric disorders (Oppositional-defiant and Conduct Disorders, Dyslexia) and 27 healthy controls.
Results showed a significant positive response to the anti-Yo antibody immunoreactivity in the Purkinje cells of the cerebellum of ADHD children, compared with the control group and the psychiatric non-ADHD children. This association points to an immune dysregulation and the involvement of the cerebellum in ADHD.

1. Introduction

It has been hypothesized that several childhood diseases are caused by autoimmune disorders. This hypothesis is supported by reports of significantly high anti-brain and, more specifically, anti-basal ganglia antibodies found in children with Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections (PANDAS) (Murphy and Barkley, 1996; Pavone et al., 2004; Singer et al., 2004) or with Tourette's syndrome, as well as in patients with Sydenham's chorea, which is a manifestation of rheumatic fever following infection by group A β-hemolytic streptococci (GABHS) (Church et al., 2002, 2003).

The immune-mediated disorder hypothesis is further supported by the demonstration that the infusion of IgG of sera from patients with PANDAS induces oral stereotypies in rats (Taylor et al., 2002) and that antibodies from a patient with Sydenham's chorea reacted against neuronal antigens at the GABHS surface (Kirvan et al., 2003).

Attention-deficit/hyperactivity disorder (ADHD) is a serious psychiatric condition that causes marked behavioral and social impairment throughout a person's life. ADHD begins in childhood and may persist into adult life in a substantial subgroup of patients (Faraone and Biederman, 2005).

The precise etiology of ADHD is not fully understood. The majority of current theories focus on dysfunction in the prefrontal brain as well as in striatal and thalamic structures (i.e. fronto-striatothalamofrontal circuits). However, in structural imaging research, cerebellar abnormalities are among the most consistently reported findings in ADHD (Semrud-Clikeman et al., 2000; Ashtari et al., 2005).

Indeed, numerous volumetric studies have reported reduced cerebellar volumes and developmental alterations in the cerebellum in children with ADHD (Berquin et al., 1998; Castellanos and Acosta, 2002; Castellanos et al., 2002; Mackie et al., 2007).

Given it close relationship with the prefrontal cortex and basal ganglia, the cerebellum is thought to play an important role in cognition, particularly in verbal working memory, implicit learning, temporal information processing as well as in shifts in attention and emotional regulation (Schmahmann and Sherman, 1998; Ivry et al., 2002; Ito, 2008). Consequently, impaired cerebellar activity may result in cerebellum cognitive and affective syndrome (CCAS).

In view of the potential involvement of this brain structure in the onset of ADHD, we conducted a series of experiments aimed at elucidating the role played by the cerebellum in children affected by ADHD.

The aim of the study was to evaluate the possible role of anti-Yo antibodies as a marker of an immune response that is directed against

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the cerebellum. Anti-Yo (PCA-1) neuronal antibodies represent some of the most commonly used markers employed in clinical practice for diagnosing paraneoplastic cerebellar degeneration (PCD). Such antibodies may also be found in ovarian cancer patients without PCD. Almost all paraneoplastic disorders are immune-mediated, thus hypothesizing an immune dysregulation (Darnell and Posner, 2003). If the association between anti-Yo antibodies and ADHD is validated, the subsequent step would be to find a causal relationship between ADHD and the presence of anti-cerebellum antibodies. Moreover, in order to evaluate the specific features of immune dysregulation in ADHD, we compared ADHD children with a control group without psychiatric disorders and with a group of children with other psychiatric disorders (Oppositional-defiant and Conduct Disorders, Dyslexia).

2. Material and methods

2.1. Subjects

We studied 30 consecutive drug-naïve Caucasian outpatients with ADHD (29 males and 1 female, mean age of 9.2 ± 2.3 years, age range of 6–17.5 years), diagnosed at the Clinic for Developmental Neurology and Psychiatry of the S. Pertini Hospital in Rome. All the ADHD subjects belonged to the combined subtype: we selected the combined subtype for the purposes of this pilot study so as to enroll the most serious cases of ADHD. Moreover, fifty percent of the ADHD children presented other psychiatric comorbidities, including Oppositional-defiant Disorder (ODD), Conduct Disorder (CD) and Dyslexia.

On the basis of the presence of comorbidities in the ADHD group, another group of children without ADHD psychiatric disorders were included in the study to be able to better define the specific features of immune dysregulation in this pathology: 19 children with a diagnosis of Oppositional-defiant or Conduct Disorders and Dyslexia (all males, mean age of 9.0 ± 2.8 years, age range of 6–15 years), were enrolled at the Clinic for Developmental Neurology and Psychiatry of the S. Pertini Hospital in Rome.

The control group was matched for sex and age and was composed of 27 healthy Caucasian sex and age-matched children (26 males and 1 female, 9.1 ± 1.8 years, age range 6–17 years), who were randomly recruited from a community-based survey and were attending two elementary and junior high schools from the same urban area of Rome.

Both the children and parents in the groups of patients with ADHD and other psychiatric disorders separately underwent a semi-structured psychiatric interview, i.e. the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL) (Kaufman et al., 1997), conducted by an experienced child psychiatrist (RD). All the children (i.e. the ADHD group, other psychiatric disorder group and control group) underwent an additional routine diagnostic assessment, based on the ADHD-Rating Scale (ADHD-RS) (DuPaul et al., 1998) adapted for the Italian population (Marzocchi and Cornoldi, 2000), which was filled out by the children's parents and school teachers.

The aim of this additional assessment was to confirm the diagnosis of ADHD according to the DSM-IV criteria in the children with ADHD and rule out the diagnosis in the children in both the other groups. On the basis of the Wechsler Intelligence Scale for Children-Revised (WISC-III) (Wechsler, 1991), all the children with an intelligence quotient (IQ) <70 were excluded.

All the children were assessed by means of a neurological evaluation: no evidence emerged of ataxia, encephalopathy or other neurological disorders. In accordance with the DSM-IV criteria, Dyslexia was assessed

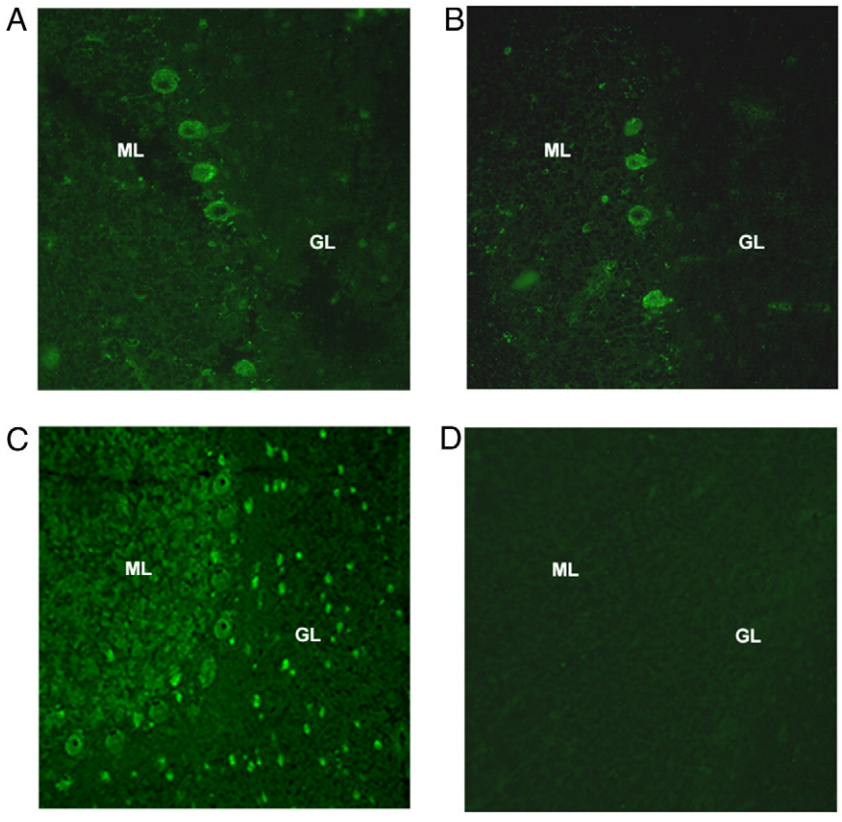

Fig. 1. Immunofluorescence reaction in ADHD patients. (A and B) Representative photomicrograph showing the positive immunofluorescence signal only for purkinje cells (anti-Yo antibodies) in ADHD children (left panel) and in neurological patient (right panel) 200X magnification. (C) Representative photomicrograph showing the positive immunorfluorescence signal in the cerebellar neurons of ADHD patient (200×). The positive reaction shows staining of Purkinje cell cytoplasm and of granular neuronal nuclei (anti-Hu antibodies). (D) Representative photomicrograph showing the negative immunorfluorescence reaction (200×). The reaction shows no staining neither of Purkinje cell cytoplasm nor in granular neuronal nuclei. ML, molecular layer; GL granular layer of cerebellum.