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Paraneoplastic syndrome-associated neuronal antibodies in adult ADHD

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ABSTRACT

A high seroprevalence of Yo antibodies targeting cerebellar Purkinje cells was recently reported in children with attention deficit/hyperactivity disorder (ADHD). We investigated the presence of 8 paraneoplastic neurological syndrome (PNS)-associated antibodies including anti-Yo in 169 adult ADHD patients. No associations between ADHD and serum Yo antibodies or other antibodies associated with PNS were found. However, 10 out of 48 ADHD patient sera analyzed by immunofluorescence presented antibodies targeting cerebellar Purkinje cells. This reactivity probably represents the presence of low levels of antibodies against multiple cellular hitherto unknown antigens with little to no clinical significance.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a neuropsychiatric disorder characterized by inattention and hyperactive/impulsive behavior and affects approximately 5–8% of children (Polanczyk et al., 2007). The disorder results in educational, occupational and social impairments, and is often associated with other psychiatric comorbidities including significant depression, anxiety, oppositional-defiant disorder and substance use disorder (Halmøy et al., 2009). ADHD often persists into adulthood and the average prevalence is estimated to be between 2.5% and 4.9% in the adult population (Simon et al., 2009; Kessler et al., 2006). Both genetic and environmental risk factors have been proposed, but the precise etiology of ADHD is still largely unknown.

Autoantibodies affecting the central nervous system have been suggested to play a role in neuropsychiatric disorders including autism, schizophrenia and limbic encephalitis (Margari et al., 2013; Moscato et al., 2010; Braunschweig and Van de Water, 2012; Ezeoke et al., 2013; Leypoldt et al., 2015). For a review see Najjar et al. (2013). Laadhar et al. detected neuronal antibodies in sera from 20 out of 103 Tunisian psychiatric patients, including 5 patients with the paraneoplastic neurological syndromes (PNS) associated antibodies anti-Ri or anti-Yo. Antibodies were not detected in any of the 41 control subjects (Laadhar et al., 2015). However, Dahm et al. recently reported antibodies against brain antigens with comparable seroprevalence in both healthy subjects (N = 1703) and patients with neuropsychiatric disorders (N = 2533), and questioned their clinical importance (Dahm et al., 2014).

An Italian study has suggested a high prevalence of cerebellar Purkinje cell specific antibodies in children with ADHD (Passarelli et al., 2013). Yo antibodies were detected in the serum of 26 out of 30 ADHD children, and in none of the 27 sex- and age-matched healthy controls. In addition, 5 of 19 non-ADHD children with oppositional-defiant or conduct disorder and dyslexia also had anti-Yo in their sera. These results are unexpected as the presence of neuronal antibodies like anti-Yo, anti-Ri, anti-Hu, anti-CRMP5, anti-Ma1/2, and anti-amphiphysin are associated with PNS, and indicate an underlying cancer (Storstein and Vedeler, 2007). For example, anti-Yo normally causes paraneoplastic cerebellar degeneration (PCD) in women with breast or ovarian cancer. However, cerebellar dysfunction has also been suspected in ADHD (S. Mackie et al. 2007).

The study by Passarelli et al. (2013) suggests that Yo antibodies may be associated with ADHD and without cancer. We therefore performed a similar study on a larger sample (N = 169) of Norwegian adult ADHD patients. We were not able to confirm an association between ADHD and neuronal antibodies related to PNS, in accordance with the study by Dahm et al. (2014) on other psychiatric phenotypes.

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2. Material and methods

2.1. Subjects

Adult Norwegian ADHD patients (N = 169, age 17–60 years) were included in this study. Adult ADHD is defined as ADHD that has appeared before age 12 and persists beyond 17 years of age (DSM-5, American Psychiatric Association, 2013). All patients were evaluated by experienced clinicians and diagnosed with persistent ADHD according to DSM-IV criteria (American Psychiatric Association, 2000) as previously described (Johansson et al., 2008; Halmøy et al., 2009; Haavik et al., 2010). Furthermore, current and past symptom scores were collected using the auto-questionnaires ASRS (Adult ADHD Self-Report Scale (Kessler et al., 2005) and WURS (Wender Utah Rating Scale (Ward et al., 1993)), in addition to a detailed questionnaire on psychiatric comorbidity and history. The ADHD subtypes were defined by 21 or more points on one or both of the ASRS subscales. 51.9% of the ADHD patients (N = 81) presented the combined subtype, 21.2% (N = 33) were predominantly inattentive, and were 5.1% (N = 8) predominantly hyperactive/impulsive. The remaining patients were subthreshold.

Healthy controls were recruited from the Medical Birth Registry of Norway (MBRN) database by invitation letters. As for the ADHD patients, all controls completed self-report questionnaires in the form of the ASRS, WURS and questionnaire on psychiatric comorbidity and history. Fifty-six responders aged 18–74 years, and from whom we received completed self-report questionnaires and biological samples, were selected.

Clinical characteristics of the ADHD patients and control subjects are summarized in Table 1. The age and gender distributions were fairly equal between the ADHD patients and the controls. The mean age of the ADHD patients was 33.0 years (SD 10.1) versus 34.9 years (SD 15.5) for the control group, and the proportion of females was 49.7% in the ADHD group and 55.4% in the control group.

Sera from patients with PNS with Yo or Hu antibodies were obtained from the Neurology research laboratory, Haukeland University Hospital, Bergen, Norway.

The project was approved by the Regional Committee for Medical Research Ethics of Western Norway (IRB 00001872; 2013/543). Written informed consent was obtained from all participants in the study.

Table 1
Clinical characteristics of ADHD patients and control subjects.

	ADHD patients	Controls	p-Value[a]
N	169	56	
Females, % (N)	49.7 (84)	55.4 (31)	0.538
Age range, years	17–60	18–74	
Age, years: mean ± SD	33.0 ± 10.1	34.9 ± 15.5	0.385[b]
ADHD treatment in childhood, % (N)[c]	11.3 (18/159)	0 (0/56)	0.004
Self-reported morbidity, % (N)[c]			
Significant depression/anxiety	63.5 (101/159)	1.8 (1/56)	<0.001
Bipolar Disorder	9.2 (14/153)	0 (0/56)	0.024
Dyslexia	49.0 (77/157)	0 (0/56)	< 0.001
Intellectual Disability	2.5 (4/159)	0 (0/56)	0.575
Epilepsy	4.4 (7/160)	0 (0/56)	0.194
Migraine	27.2 (43/158)	14.3 (8/56)	0.067
ADHD variables, mean ± SD (N)[d]			
ASRS inattentive score	23.7 ± 6.4 (157)	11.3 ± 4.4 (52)	<0.001[b]
ASRS hyperactive/impulsive score	21.8 ± 6.7 (159)	8.9 ± 4.3 (53)	<0.001[b]
ASRS total score	45.4 ± 11.8 (156)	20.2 ± 8.0 (52)	<0.001[b]
WURS total	57.7 ± 16.7 (143)	12.0 ± 8.3 (54)	<0.001[b]
ASRS subtype, % (N)			
Combined	51.9 (81)	1.9 (1)	
Hyperactive/Impulsive	5.1 (8)	0 (0)	
Inattentive	21.2 (33)	1.9 (1)	
Subthreshold	21.8 (34)	96.2(50)	
Sum	(156)	(52)	

ASRS = Adult ADHD Self-Report Scale, WURS = Wender Utah Rating Scale.
[a] Fisher's exact test with 2-sided p-values if not otherwise stated.
[b] T-test with 2-sided p-values.
[c] Positive/case numbers. The varying case numbers are due to blank responses on the questionnaires.
[d] Case numbers.

2.2. Detection of autoantibodies

2.2.1. Immunoblotting

The 169 ADHD sera were tested for antibodies by two immunoblot protocols, the PNS + 2 Line Blot from *Ravo* Diagnostika GmbH (Freiburg, Germany) and the Neuronal Antigens Profile 2 from Euroimmun Medizinische Labordiagnostika AG (Lübeck, Germany). Sera from PNS patients with Yo and Hu antibodies, in addition to Hu positive Profile 2 Kit control serum (Euroimmun), were used as positive controls. Sera from the 56 normal controls were also analyzed by the Neuronal Antigens Profile 2 strips. Line blots with no added serum samples were used as negative controls.

Nitrocellulose test strips (PNS + 2 Line Blot from *Ravo* Diagnostika coated with the recombinant antigens HuD, CDR2/Yo, Ri (Nova1), CRMP5, amphiphysin, Ma1/Ma2, SOX1 and GAD65 were incubated with a 1:2000 dilution of patient- and control sera according to the manufacturer's instructions. Bound antibodies were detected by alkaline phosphate conjugated to anti-human IgG using BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium) as substrate. The intensity of the bands was evaluated manually. A distinct band corresponding to the actual position of the antigen in the line blot was considered as a positive signal.

Euroline line blots (Neuronal Antigens Profile 2 from Euroimmun Medizinische Labordiagnostika AG) coated with recombinant amphiphysin, CRMP5, PNMA2 (Ma2/Ta), Ri (Nova1), CDR2/Yo, and HuD were utilized according to the protocol provided by the supplier. Patient- and control sera were diluted 1:101, added to the line blots and assayed in a EuroBlotMaster 44 blot processor. Incubated line blots were scanned and automatically evaluated with the EuroLineScan software (EuroLineScanV3.4.4). All sera were analyzed in duplicate.

2.2.2. Indirect immunofluorescence

Rat cerebellum was fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer for 48 h, rinsed in 0.1 M phosphate buffer, cryoprotected by 20% sucrose in 0.1 M phosphate buffer and snap-frozen. Cryostat sections (8 μm thick) of fixed tissue on microscope slides were incubated with sera diluted 1:100 and 1:500 in PBT (PBS with 1% BSA and 0.2% Triton X) at 4 °C overnight in a moist chamber. The slides were subsequently washed with PBT and incubated with Alexa Fluor 488 goat anti-human IgG (Molecular probes, Eugene, OR, USA) diluted 1:100 in PBT, for 90 min at room temperature. Finally, the slides were washed in PBS, covered and examined by fluorescence microscopy (Leica DM IL, Leica Microsystems Ltd., Heerbrugg, Switzerland, or Nikon Eclipse E800, Nikon Corporation Instruments Company).

Euroimmun Indirect Immunofluorescence test (Euroimmun #FA 1111–monkey cerebellum) was performed according to the manufacturer's protocol (Euroimmun Medizinische Labordiagnostika AG) to detect serum antibodies. Frozen sections of primate cerebellum were incubated for 30 min at room temperature with a 1:10 dilution of patient- or control serum in PBS-Tween. Attached antibodies were stained with fluorescein-labeled anti-human IgA/G/M antibodies. The slides were evaluated using a Nikon Eclipse E800 fluorescence microscope. Sera from PNS patients with Yo and Hu antibodies, in addition to Yo Ab control (Euroimmun, CA-1113-0101), were used as positive controls. Sections with no serum samples, but only fluorescein-labeled anti-human IgA/G/M were used as negative controls.

2.2.3. Cell culture and transfection

HeLa cells (ATCC-CCL-2) were grown in DMEM (Dulbecco's modified eagle medium), supplemented with 10% FCS (Sigma Aldrich, St.

Louis, MO, USA), L-glutamine (PAA, M11–004) and Pen Strep (Sigma Aldrich; P4333). Cells were grown on poly-L-lysine coated glass-coverslips (10 mm) the day before transfection. cDNA for full-length CDR2 (Origene, Germany; CDR2 sequence was transferred from vector RG204900 with C-terminal GFP into vector PS10019 with N-terminal GFP) or CDR2L (Origene; CDR2L sequence transferred from vector RC206909 with C-terminal myc-DDK tag into vector PS10010 with C-terminal GFP) were used to transfect HeLa cells following the standard protocol for Lipofectamine2000 (Invitrogen Life Technologies, CA, USA; 11668-019). Transfected HeLa cells were fixed and immunocytochemically labeled with patient sera and antibodies against CDR2 and CDR2L 24 h after transfection as described elsewhere (Bittins et al., 2010). All sera and antibodies were diluted in blocking solution (0.2% gelatin in PBS). Patient sera were diluted 1:100. All samples were prepared in duplets. Secondary antibodies coupled to Alexa Fluor (AF) 594 (Invitrogen; anti-rabbit-AF594 A11012, anti-human-AF594 A11014) were used at a 1:200 dilution. Coverslips were mounted with ProLong Gold with DAPI (Invitrogen; P36931) and dried overnight. Imaging of HeLa cells was performed with a Zeiss Axiovert M200 microscope equipped with a Till Photonics Polychrome V monochromator, a sensicam imago QE CCD camera (PCO) and Till Vision 4.0 software.

2.2.4. Western blot analysis

Rat cerebellum tissue lysates were made using Chemicon Total Protein Extraction Kit #2140 (Chemicon International, Temecula, CA, USA), and stored at −80 °C. The protein concentration was determined by BioRad DC protein assay #500-0111 (Bio-Rad, CA, USA).

Lysates (30 μg) from homogenized rat cerebellar tissue were subjected to 4–12% Bis-Tris precast NuPAGE (Novex by Life, MA, USA; #NP0323), electroblotted onto nitrocellulose membrane (0.45 μm pore size; Novex by Life; #LC2001) and incubated overnight at 4 °C with diluted patient sera (1:100 and 1:500 in 0.05% PBS-Tween and SuperBlock Blocking buffer (Thermo Scientific, MA, USA; #37515)). The blots were developed using rabbit anti-human horseradish peroxidase (HRP) conjugated antibody (DAKO, Agilent Technologies, Santa Clara, CA, USA; #P0212; dilution 1:1000, incubated overnight at 4 °C) and SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific; #34080), and read in a Fujifilm LAS-1000 Plus image analyzer (ImageReader software LAS10000 pro v2.6).

2.3. Statistics

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20, IBM Corp., Armonk, NY, USA.

3. Results

3.1. Immunoblotting

None of the ADHD or control sera reacted with the PNS + 2 Line Blot. The EuroLineScan software reported several borderline signals (intensity 6–10) including CDR2/Yo, amphiphysin and/or Ma2 bands in 9 of the ADHD sera. One of the sera revealed a very weak positive CDR2/Yo band (intensity 11) as compared to the positive control bands (intensity 99–134) (data not shown).

3.2. Indirect immunofluorescence

The 10 serum samples that yielded borderline positive antibody signals and 38 randomly selected ADHD serum samples were further investigated by indirect immunofluorescence on monkey and rat cerebellar sections. Ten of the 48 (21%) ADHD sera, of which 7 gave borderline signals on Neuronal Antigens Profile 2, showed positive cytoplasmic staining of the monkey and rat Purkinje cells. The staining pattern resembled that seen with Yo antibody positive sera from patients with PCD (Fig. 1). None of the 48 normal control sera stained the Purkinje cells.

3.3. Cell culture and transfection

To investigate whether the cytoplasmic Purkinje cell staining or borderline Neuronal Antigens Profile 2 signals were due to binding to the Yo antigen (CDR2 and/or CDR2L proteins), we tested the 13 ADHD sera (10 borderline value at Euroimmun line blot and 3 line blot negative but immunofluorescence positive sera) on HeLa cells transfected with either *CDR2* or *CDR2L*. This technique has previously been used to determine the presence of CDR2 and/or CDR2L antibodies in human serum samples (Eichler et al., 2013). None of the 13 ADHD patient sera bound to the transfected CDR2 or CDR2L cells (data not shown). The anti-Yo positive control serum from a patient with PCD bound to both the CDR2 and CDR2L transfected cells.

3.4. Western blot analysis

Western blot analysis was performed using 4 of the ADHD patient sera that stained Purkinje cell cytoplasm by immunofluorescence and also yielded borderline positive antibody signals revealed by the Euroimmun line blot. As positive and negative controls we used serum from a PCD patient with Yo antibodies and only HRP conjugated antibody without serum, respectively.

The 4 ADHD patient sera reacted against several proteins with molecular weight (MW) of 10 to 83 kDa in the rat cerebellum extract, but with no binding to the CDR2/CDR2L antigens with a MW of 62 kDa. The Yo positive serum also produced several bands, but showed strong bands in the 62 kDa (CDR2/2L) region (Fig. 2). The HRP conjugated antibody alone did not stain the blots.

4. Discussion

Neuronal antibodies have previously been reported in sera from patients with various neuropsychiatric disorders such as autism, schizophrenia and limbic encephalitis (Laadhar et al., 2015; Mostafa et al., 2014; Margari et al., 2013; Moscato et al., 2010; Braunschweig and Van de Water, 2012; Ezeoke et al., 2013; Leypoldt et al., 2015). However, there are several questions regarding serum antibodies in neuropsychiatric disorders; why do they arise, do they pass the blood–brain barrier, are they causative for the disease, are they apparent only at disease debut or also later in the disease progression, is the antibody production affected by psychiatric drug treatment etc. In addition, replication studies are scarce (Dahm et al., 2014; Ezeoke et al., 2013).

Serum antibodies directed against antigens like Ri (*NOVA1* and *NOVA2* genes), Ma2/Ta (*PNMA2* gene), CRMP5 (*DPYSL5* gene), HuD (*ELAVL4* gene), Yo (*CDR2* and *CDR2L* genes), and amphiphysin (*AMPH* gene) are associated with PNS such as encephalomyelitis, limbic encephalitis, peripheral neuronopathy and cerebellar ataxia. We have previously shown that the majority of patients with paraneoplastic cerebellar degeneration have Yo antibodies against both CDR2 and CDR2L proteins (Eichler et al., 2013). The presence of antibodies against the CDR2 and CDR2L proteins are associated with loss of cerebellar Purkinje cells, probably due to dysregulation of the cell calcium homeostasis (Schubert et al., 2014).

Recently it was shown that ADHD patients had a high prevalence of serum Yo antibodies (Passarelli et al., 2013). The sera gave a cytoplasmic staining of the cerebellar Purkinje cells and moderate signals at Euroimmun Euroline line blots (Neuronal Antigens Profile 2) indicating the presence of CDR2/Yo antibodies. We found these results unexpected since Yo antibodies usually are markers of PCD in postmenopausal women with ovarian or breast cancer (Storstein and Vedeler, 2007).

In this study, we examined 169 adult ADHD patients for the occurrence of PNS associated antibodies. None of the sera were positive

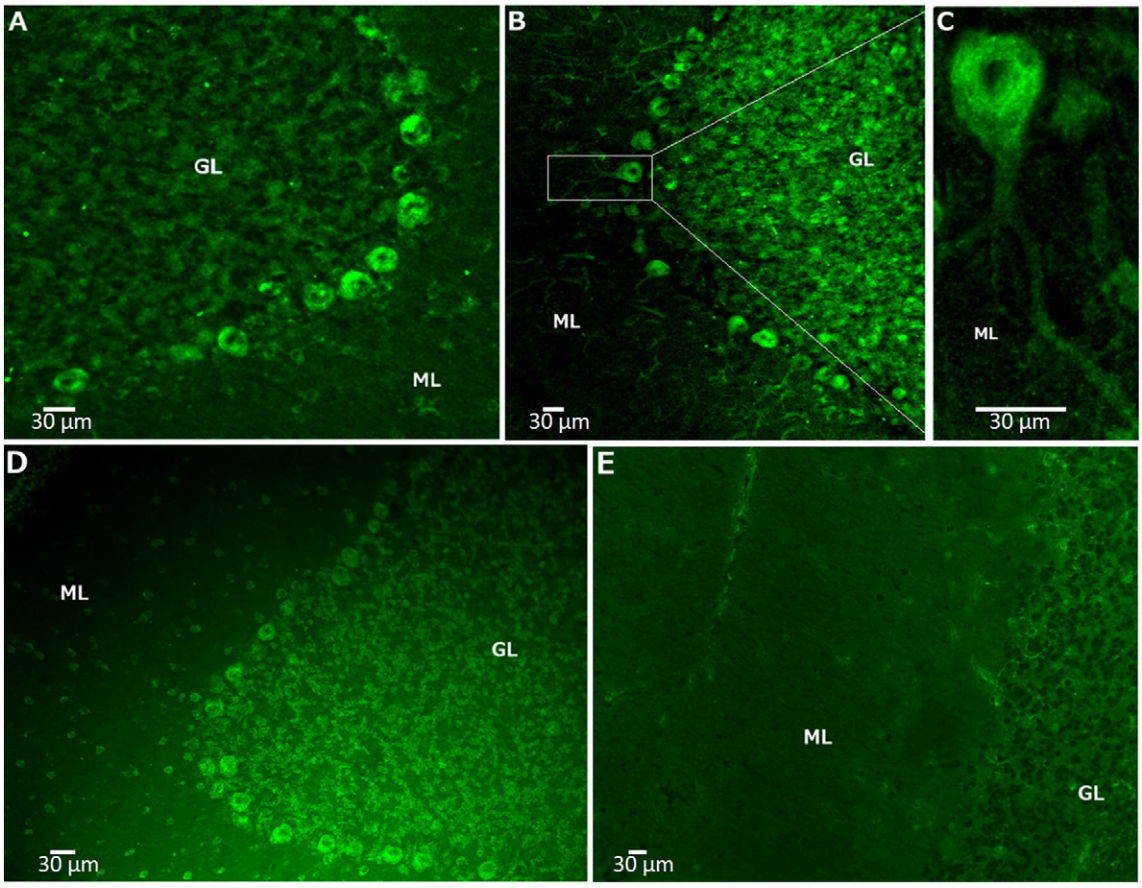

Fig. 1. Indirect immunofluorescence on cerebellar sections. Sera from two representative ADHD patients (A and B/C) showing positive cytoplasmic Purkinje cell staining at 1:100 dilution, (A–B) 20× and (C) 60× (serum A corresponds to lane 2 serum, and serum B/C corresponds to lane 3 serum respectively in Fig. 2). (D) Yo antibody positive serum at 1:500 dilution from a PCD patient (20×), and (E) control serum from a non-ADHD individual (1:100 dilution; 20×). No staining of the Purkinje cell bodies (layer between GL and ML) was observed in (E). GL = granular cell layer, ML = molecular layer.

in the line blots from Ravo Diagnostika when interpreted manually following our diagnostic PNS protocol, whereas 1 of the sera revealed a very weak positive CDR2/Yo band and 9 sera showed borderline values for CDR2/Yo, amphiphysin and/or Ma2 in the EuroLineScan evaluated Euroimmun line blots. The various results from Ravo- and Euroimmun line blots may be due to the use of different patient sera dilutions (1:2000 vs 1:101, respectively), but also to different evaluation techniques.

We also tested 48 ADHD sera, including the 10 borderline value sera in the Euroimmun line blot, by indirect immunofluorescence using cerebellum sections. Ten sera showed positive cytoplasmic staining of the Purkinje cells and could resemble anti-Yo staining, including 7 of the 10 borderline value sera.

The Yo bands of both Ravo Diagnostica and Euroimmun line blots were based on recombinant CDR2 and not CDR2L, and serum antibodies against CDR2L cannot be excluded. However, we did not detect Yo antibodies in the 13 ADHD sera tested on cells transfected with either CDR2 or CDR2L. Furthermore, we tested 4 of these sera using Western blot of cerebellar extract. As expected, the sera reacted with various proteins, but not with the CDR2/CDR2L proteins (Fig. 2).

In the previous report by Passarelli et al. (2013) sera from 26 of 30 (87%) children with ADHD reacted with Purkinje cells. These results are not in line with ours where we find no evidence for Yo antibodies in adult ADHD patients. However, we cannot exclude that there may be a different prevalence of such antibodies in adult and young ADHD patients. However, this explanation is less likely, as we did not observe an effect of age on the borderline neuronal antibody levels detected by the Euroimmun line blot in our 10 patients (age 20–43 years) and age-dependent decline in antibody levels are not commonly observed in autoimmune disorders (Manz et al., 2005).

In conclusion, our study did not find neuronal PNS antibodies such as anti-Yo in sera of adult ADHD patients. However, some of the patients had antibodies against other proteins in the Purkinje cells. Further studies are necessary to reveal what proteins these sera react with and the possible relevance of Purkinje cell antibodies in ADHD. The presence of low-levels of serum antibodies must be interpreted with caution. If these antibodies can also be shown intrathecally, this may indicate a possible association with the disease. However, as such studies are still lacking for ADHD, it is not possible to conclude that such serum antibodies have a pathogenic role in this disease.

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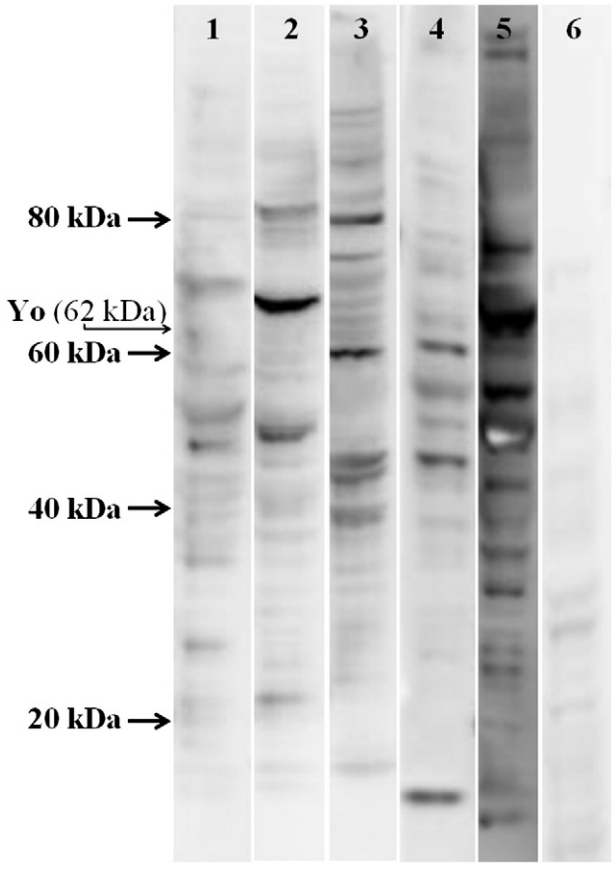

Fig. 2. Western blot analysis of rat cerebellum tissue lysate. Lanes 1 through 4 represents four different ADHD patient sera. Lane 5: serum from a Yo positive PCD patient, lane 6: control serum from a non-ADHD individual. All sera used at 1:100 dilutions. Only the PCD patient serum (lane 5) demonstrated strong staining of the Yo antigen (62 kDa band).

References

American Psychiatric Association (APA), 2000. Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR. 4th ed. Washington DC.

American Psychiatric Association (APA), 2013. DSM-5. 5th ed. Washington DC.

Bittins, C., Eichler, T., Hammer, J., Gerdes, H.-H., 2010. Dominant-negative myosin Va impairs retrograde but not anterograde axonal transport of large dense core vesicles. Cell. Mol. Neurobiol. 30, 369–379.

Braunschweig, D., Van de Water, J., 2012. Maternal autoantibodies in autism. Arch. Neurol. 69 (6), 693–699.

Dahm, L., Ott, C., Steiner, J., Stepniak, B., Teegen, B., Saschenbrecker, S., Hammer, C., Borowski, K., Begemann, M., Lemke, S., Rentzsch, K., Probst, C., Martens, H., Wienands, J., Spalletta, G., Weissenborn, K., Stöcker, W., Ehrenreich, H., 2014. Seroprevalence of autoantibodies against brain antigens in health and disease. Ann. Neurol. 76 (1), 82–94.

Eichler, T.W., Totland, C., Haugen, M., Qvale, T.H., Mazengia, K., Storstein, A., Haukanes, B.I., Vedeler, C.A., 2013. CDR2L antibodies: a new player in paraneoplastic cerebellar degeneration. PLoS One 8 (6). http://dx.doi.org/10.1371/journal.pone.0066002.

EuroLineScanV3.4.4. http://www.euroimmun.com/fileadmin/template/images/pdf/DV_0000_I_UK_G08.pdf

Ezeoke, A., Mellor, A., Buckley, P., Miller, B., 2013. A systematic, quantitative review of blood autoantibodies in schizophrenia. Schizophr. Res. 150 (1), 245–251.

Haavik, J., Halmøy, A., Lundervold, A.J., Fasmer, O.B., 2010. Clinical assessment and diagnosis of adults with attention-deficit/hyperactivity disorder. Expert. Rev. Neurother. 10 (10), 1569–1580.

Halmøy, A., Fasmer, O.B., Gillberg, C., Haavik, J., 2009. Occupational outcome in adult ADHD: impact of symptom profile, comorbid psychiatric problems, and treatment: a cross-sectional study of 414 clinically diagnosed adult ADHD patients. J. Atten. Disord. 13 (2), 175–187.

Johansson, S., Halleland, H., Halmøy, A., Jacobsen, K.K., Landaas, E.T., Dramsdahl, M., Fasmer, O.B., Bergsholm, P., Lundervold, A.J., Gillberg, C., Hugdahl, K., Knappskog, P.M., Haavik, J., 2008. Genetic analysis of dopamine related genes in adult ADHD patients suggest an association with the DRD5-microsatellite repeat, but not with DRD4 or SLC6A3 VNTRs. Am. J. Med. Genet. B Neuropsychiatr. Genet. 147 B (8), 1470–1475.

Kessler, R.C., Adler, L., Ames, M., Demler, O., Faraone, S., Hiripi, E., Howes, M.J., Jin, R., Secnik, K., Spencer, T., Ustun, T.B., Walters, E.E., 2005. The World Health Organization Adult ADHD Self-Report Scale (ASRS): a short screening scale for use in the general population. Psychol. Med. 35, 245–256.

Kessler, R.C., Adler, L., Barkley, R., Biederman, J., Conners, C.K., Demler, O., Faraone, S.V., Greenhill, L.L., Howes, M.J., Secnik, K., Spencer, T., Ustun, T.B., Walters, E.E., Zaslavsky, A.M., 2006. The prevalence and correlates of adult ADHD in the United States: results from the National Comorbidity Survey Replication. Am. J. Psychiatry 163, 716–723.

Laadhar, L., Sidhom, O., Zitouni, M., Sassi, N., Abdelghaffar, W., Lahmar, H., Kallel-Sellami, M., El Hechmi, Z., Makni, S., 2015. High prevalence of antineuronal antibodies in Tunisian psychiatric inpatients. J. Neuropsychiatr. Clin. Neurosci. 27 (1), 54–58.

Leypoldt, F., Armangue, T., Dalmau, J., 2015. Autoimmune encephalopathies. Ann. N. Y. Acad. Sci. 1338 (1), 94–114.

Mackie, S., Shaw, P., Lenroot, R., Pierson, R., Greenstein, D.K., Nugent, T.F., Sharp, W.S., Giedd, J.N., Rapoport, J.L., 2007. Cerebellar development and clinical outcome in attention deficit hyperactivity disorder. Am. J. Psychiatry 164, 647–655.

Manz, R.A., Hauser, A.E., Hiepe, F., Radbruch, A., 2005. Maintenance of serum antibody levels. Annu. Rev. Immunol. 23, 367–386.

Margari, F., Petruzzelli, M.G., Mianulli, R., Toto, M., Pastore, A., Bizzaro, N., Tampoia, M., 2013. Anti-brain autoantibodies in the serum of schizophrenic patients: a case–control study. Psychiatry Res. 210 (3), 800–805.

Moscato, E.H., Jain, A., Peng, X., Hughes, E.G., Dalmau, J., Balice-Gordon, R.J., 2010. Mechanisms underlying autoimmune synaptic encephalitis leading to disorders of memory, behavior and cognition: insights from molecular, cellular and synaptic studies. Eur. J. Neurosci. 32 (2), 298–309.

Mostafa, G.A., El-Sherif, D.F., Al-Ayadhi, L.Y., 2014. Systemic auto-antibodies in children with autism. J. Neuroimmunol. 272 (1–2), 94–98.

Najjar, S., Pearlman, D.M., Alper, K., Najjar, A., Devinsky, O., 2013. Neuroinflammation and psychiatric illness. J. Neuroinflammation 10, 43.

Passarelli, F., Donfrancesco, R., Nativio, P., Pascale, E., Di Trani, M., Patti, A.M., Vulcano, A., Gozzo, P., Villa, M.P., 2013. Anti-Purkinje cell antibody as a biological marker in attention deficit/hyperactivity disorder: a pilot study. J. Neuroimmunol. 258 (1–2), 67–70.

Polanczyk, G., de Lima, M.S., Horta, B.L., Biederman, J., Rohde, L.A., 2007. The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am. J. Psychiatry 164 (6), 942–948.

Schubert, M., Panja, D., Haugen, M., Bramham, C.R., Vedeler, C.A., 2014. Paraneoplastic CDR2 and CDR2L antibodies affect Purkinje cell calcium homeostasis. Acta Neuropathol. 128 (6), 835–852.

Simon, V., Czobor, P., Bálint, S., Mészáros, A., Bitter, I., 2009. Prevalence and correlates of adult attention-deficit hyperactivity disorder: meta-analysis. Br. J. Psychiatry 194 (3), 204–211.

Storstein, A., Vedeler, C.A., 2007. Paraneoplastic neurological syndromes and onconeural antibodies: clinical and immunological aspects. Adv. Clin. Chem. 44, 143–185.

Ward, M.F., Wender, P.H., Reimherr, F.W., 1993. The Wender Utah Rating Scale: an aid in the retrospective diagnosis of childhood attention deficit hyperactivity disorder. Am. J. Psychiatry 150 (6), 885–890.