

Autoantibodies targeting neurotransmitter biosynthetic enzymes in attention-deficit/hyperactivity disorder (ADHD)

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Dear Editors,

In a recent study of children with autism or ADHD, it was reported that 4 out of 15 ADHD patients had serum autoantibodies targeting glutamate decarboxylase 65 (GAD65) [1], one of two enzymes that convert glutamate to the inhibitory neurotransmitter GABA (for a review on GAD65 antibodies see [2]). Moreover, serum from eight of the ADHD children reacted with GABAergic Purkinje cells in sections of mouse cerebellum and three reacted with cells of the molecular and granular cell layers of the cerebellum. This was in contrast to the control sera of which none reacted.

As the patient sample group of Rout et al. [1] was small, we wanted to replicate their findings on the GAD65 antibody prevalence in a larger sample. We also examined for autoantibodies against three related neurotransmitter biosynthetic enzymes which also have been implicated in

ADHD and other neuropsychiatric disorders: aromatic-L-amino-acid decarboxylase (DOPA decarboxylase; AADC) [3, 4], tryptophan hydroxylase 1 (TPH1) [3, 5], and tyrosine hydroxylase (TH) [3, 6]. An autoimmune subtype of ADHD could explain why some patients have associated neurological symptoms and the striking differences in rates of disease progression and could lead to the development of new therapeutic strategies.

The patient recruitment protocol has been described previously [7]. From a collection of 350 ADHD serum samples, we randomly picked samples from 79 ADHD patients for antibody assays. Serum samples from 10 anonymous healthy blood donors were pooled and used as negative control and high antibody titre sera from four patients with autoimmune polyendocrine syndrome type 1 (APS-1) [8–12] were used as positive controls, one APS-1 patient for each antigen. The medical histories of the ADHD patients were obtained by auto-questionnaires and telephone interviews. Project approval was granted by the Regional Committee for Medical Research Ethics of Western Norway (IRB 00001872). An informed consent form was obtained from all ADHD and APS-1 patients.

In vitro transcription and translation of ³⁵S-radiolabeled GAD65, AADC, TPH1 and TH was performed with the TNT T3 coupled reticulocyte lysate system (Promega Corporation, Madison, WI, USA). For experimental details, see [12]. All assays for each antibody contained identical positive and negative control sera which were used to normalise between assays if a prominent phase shift was detected.

As shown in Fig. 1, 1 out of 79 ADHD patients had clearly detectable GAD65 antibodies, with an antibody reactivity of approximately 66 % of the positive control serum from an APS-1 patient. This female patient in her 40s reported to have had ADHD symptoms from an early

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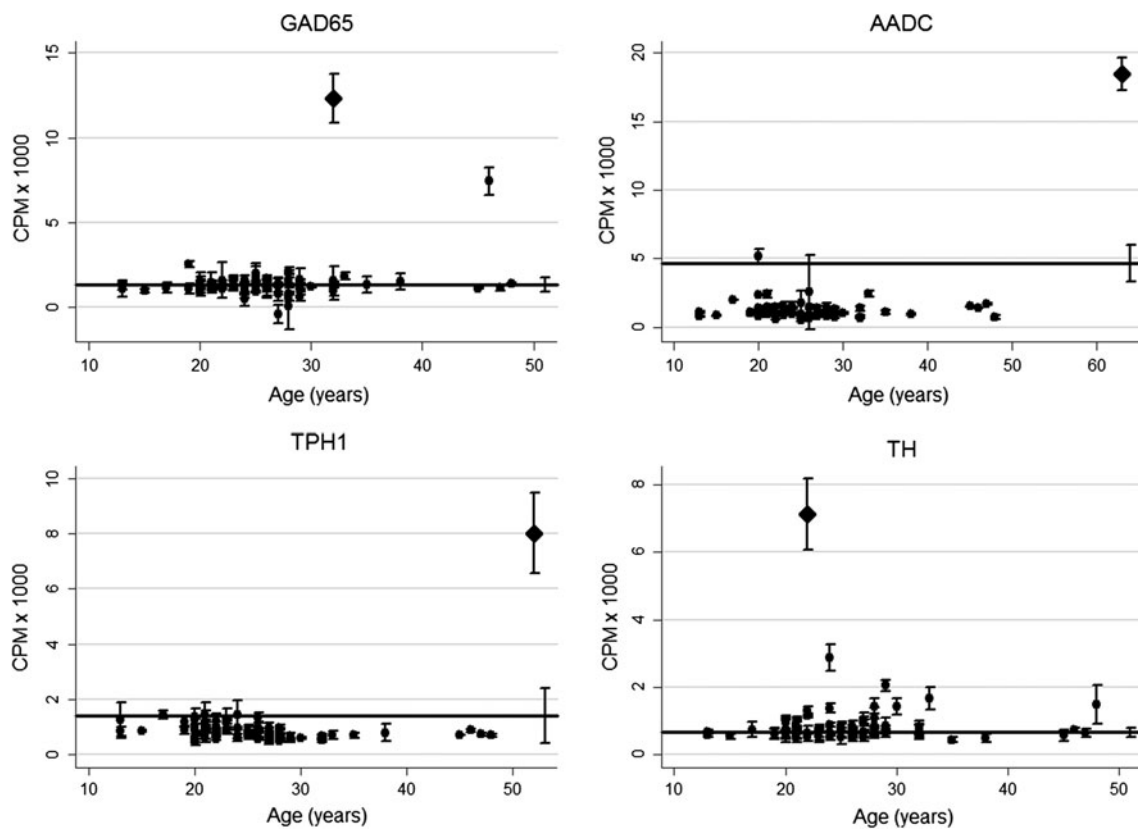


Fig. 1 Antibody reactivity (counts per minute) against the antigens. X-axis is age. Circles indicate the mean of counts per minute observed for the experiments, vertical bars are standard deviations. Positive APS-1 controls are shown as black diamonds. Horizontal bars represent

the negative controls with their standard deviations presented as the vertical bars furthest to the right. Each of the values represents the average of 2–6 experiments, except the control values which represent the average of 5–12 experiments. Made with Stata 12

age, but was formally diagnosed with ADHD as an adult. She did not have epilepsy, diabetes mellitus or known autoimmune disorders.

The ADHD patient with antibodies against AADC, with a reactivity of about 28 % of the positive APS-1 control, is a young male. He was diagnosed with ADHD as a child and also suffers from epilepsy.

None of the 79 ADHD patients had detectable TPH1 autoantibodies.

The patient with the highest antibody reactivity against TH, at approximately 40 % of the positive control serum, is a female patient in her mid 20s who was diagnosed with ADHD as an adult. She did not suffer from epilepsy, diabetes mellitus or any other known autoimmune disorders.

In summary we were not able to replicate the findings of Rout et al. [1] on the prevalence of GAD65 autoantibodies in ADHD, as we detected only one GAD65 antibody positive patient in our sample of 79 clinically diagnosed ADHD patients. This frequency is in line with previous studies of GAD65 antibodies in the presumed healthy population [13]. Therefore, we find it unlikely that GAD65 antibodies are frequently observed or involved in the pathophysiology of ADHD. As we have used an

established, validated assay protocol that has been used in routine diagnostic and research settings for many years [14], we expect that autoantibody positive sera would have been detected in our experiments.

A limitation of our findings is that the patients investigated have a different age (mean 26 vs. 9 years) and sex (48 % female vs. 20 % female) composition than the patients of Rout et al. [1]. It is thought that the levels of GAD65 antibodies in the healthy population reach a top during childhood and subsequently falls as the individual ages [13]. In addition there are known sex differences in the prevalence of several of the autoimmune disorders [15]. Although, theoretically, the prevalence of GAD65 autoantibodies could be completely different in child and adult ADHD patients, this is not consistent with findings in other autoimmune disorders [16].

As the exact prevalence of AADC, TPH1 and TH autoantibodies in the general Norwegian population is not known, we cannot conclude whether the antibody frequencies in ADHD are different from controls. However, the prevalence of AADC, TPH1 and TH antibody positive ADHD patients in our material was low. In addition, the levels of antibodies in the positive ADHD patients were

low compared to the positive controls with known autoimmune disease. Consequently we do not believe that AADC, TPH1 and TH autoantibodies are causally linked to ADHD, but as with GAD65, we cannot exclude the possibility of autoimmunity being involved in the aetiology in a minor subgroup of the patients.

Rout et al. [1] sought to confirm the presence of human antibodies against GAD65 and other potential ADHD-related antigens with immunofluorescence staining of mouse cerebellum sections with ADHD patient sera. Although the staining patterns may be due to disease specific autoantibodies, this method has several potential pitfalls. At serum dilutions at 1:500, naturally occurring human antibodies might be capable of staining cells from foreign species. Consequently, the results of Rout et al. [1] could be due to a preformed antibody reaction, for example targeting the carbohydrate residue α Gal, synthesised by *N*-acetylglucosaminidase α -1,3-galactosyltransferase (Ggta1) [17, 18].

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Conflict of interest The authors declare that they have no conflict of interest.

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References

- Rout UK, Mungan NK, Dhossche DM (2012) Presence of GAD65 autoantibodies in the serum of children with autism or ADHD. *Eur Child Adolesc Psychiatry* 21(3):141–147. doi:10.1007/s00787-012-0245-1
- Ali F, Rowley M, Jayakrishnan B, Teuber S, Gershwin ME, Mackay IR (2011) Stiff-person syndrome (SPS) and anti-GAD-related CNS degenerations: protean additions to the autoimmune central neuropathies. *J Autoimmun* 37(2):79–87. doi:10.1016/j.jaut.2011.05.005
- Haavik J, Blau N, Thöny B (2008) Mutations in human monoamine-related neurotransmitter pathway genes. *Hum Mutat* 29(7):891–902. doi:10.1002/humu.20700
- Ribasés M, Ramos-Quiroga JA, Hervas A, Bosch R, Bielsa A, Gastaminza X, Artigas J, Rodríguez-Ben S, Estivill X, Casas M, Cormand B, Bayes M (2009) Exploration of 19 serotonergic candidate genes in adults and children with attention-deficit/hyperactivity disorder identifies association for 5HT2A, DDC and MAOB. *Mol Psychiatry* 14(1):71–85. doi:10.1038/sj.mp.4002100
- Halmøy A, Johansson S, Winge I, McKinney JA, Knappskog PM, Haavik J (2010) Attention-deficit/hyperactivity disorder symptoms in offspring of mothers with impaired serotonin production. *Arch Gen Psychiatry* 67(10):1033–1043. doi:10.1001/archgenpsychiatry.2010.124
- Perez-Costas E, Melendez-Ferro M, Rice MW, Conley RR, Roberts RC (2012) Dopamine pathology in schizophrenia: analysis of total and phosphorylated tyrosine hydroxylase in the substantia nigra. *Front Psychiatry Front Res Found* 3:31. doi:10.3389/fpsy.2012.00031
- Johansson S, Halleland H, Halmøy A, Jacobsen KK, Landaas ET, Dramsdahl M, Fasmer OB, Bergsholm P, Lundervold AJ, Gillberg C, Hugdahl K, Knappskog PM, Haavik J (2008) Genetic analyses 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 Part B Neuropsychiatr Genet* 147B(8):1470–1475. doi:10.1002/ajmg.b.30662
- Tuomi T, Björnsen P, Falorni A, Partanen J, Perheentupa J, Lernmark A, Miettinen A (1996) Antibodies to glutamic acid decarboxylase and insulin-dependent diabetes in patients with autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab* 81(4):1488–1494. doi:10.1210/jc.81.4.1488
- Bratland E, Magitta NF, Boe Wolff AS, Ekern T, Knappskog PM, Kämpe O, Haavik J, Husebye ES (2012) Autoantibodies against aromatic amino acid hydroxylases in patients with autoimmune polyendocrine syndrome type 1 target multiple antigenic determinants and reveal regulatory regions crucial for enzymatic activity. *Immunobiology*. doi:10.1016/j.imbio.2012.10.006
- Ekwall O, Hedstrand H, Grimelius L, Haavik J, Perheentupa J, Gustafsson J, Husebye E, Kämpe O, Rorsman F (1998) Identification of tryptophan hydroxylase as an intestinal autoantigen. *Lancet* 352(9124):279–283. doi:10.1016/s0140-6736(97)11050-9
- Hedstrand H, Ekwall O, Haavik J, Landgren E, Betterle C, Perheentupa J, Gustafsson J, Husebye E, Rorsman F, Kämpe O (2000) Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome type I. *Biochem Biophys Res Commun* 267(1):456–461. doi:10.1006/bbrc.1999.1945
- Husebye ES, Gebre-Medhin G, Tuomi T, Perheentupa J, Landin-Olsson M, Gustafsson J, Rorsman F, Kämpe O (1997) Autoantibodies against aromatic L-amino acid decarboxylase in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab* 82(1):147–150. doi:10.1210/jc.82.1.147
- Kordonouri O, Hartmann R, Grüters-Kieslich A, Knip M, Danne T (2002) Age-specific levels of diabetes-related GAD and IA-2 antibodies in healthy children and adults. *J Pediatr Endocrinol Metab JPEM* 15(1):47–52. doi:10.1515/JPEM.2002.15.1.47
- Törn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ (2008) Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. *Diabetologia* 51(5):846–852. doi:10.1007/s00125-008-0967-2
- Whitacre CC (2001) Sex differences in autoimmune disease. *Nat Immunol* 2(9):777–780. doi:10.1038/ni0901-777
- Manz RA, Hauser AE, Hiepe F, Radbruch A (2005) Maintenance of serum antibody levels. *Annu Rev Immunol* 23:367–386. doi:10.1146/annurev.immunol.23.021704.115723
- Li S, Waer M, Billiau AD (2009) Xenotransplantation: role of natural immunity. *Transpl Immunol* 21(2):70–74. doi:10.1016/j.trim.2008.10.004
- Sugino K, Hempel CM, Miller MN, Hattox AM, Shapiro P, Wu C, Huang ZJ, Nelson SB (2006) Molecular taxonomy of major neuronal classes in the adult mouse forebrain. *Nat Neurosci* 9(1):99–107. doi:10.1038/nn1618