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Detection of auto-antibodies to DAT in the serum: Interactions with DAT genotype and psycho-stimulant therapy for ADHD

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ABSTRACT

Interest is rising for auto-immune contribution in neuro-psychiatry. We evaluated the auto-antibodies against dopamine transporter (DAT aAbs) in 61 children (46 ADHD who met DSM-IV-TR criteria, 15 healthy controls).
Methods: ADHD patients were assigned, according to severity, either to a non-pharmacological therapy (NPT, N = 32) or to a pharmacological treatment (PT, N = 14) with methylphenidate (MPH). In ADHD children, blood samples were withdrawn twice, at recruitment (T0 basal) and after 6 weeks (T1); following 16 excluded subjects, DAT genotype was characterized (9-repeat or 10-repeat alleles; N = 15 each). After 18 months of NPT or PT, some patients (carrying at least one 9-repeat allele) were blood sampled again (T2), for comparison with healthy controls (final n = 8)
Results: Compared to NPT, basal DAT aAbs titers were higher within most severe patients (then assigned to PT), specifically if carrying a DAT 10/10 genotype. DAT aAbs levels of NPT group resulted highly correlated with distinct subscales of Conners' Parent/Teacher Scales (Rs > 0.34), especially within DAT 10/10 genotype (Rs > 0.53). While T1 titers were elevated over T0 baseline for NPT children, such an increase was not observed in PT patients carrying at least one 9-repeat allele, who also showed behavioral response to subchronic MPH. After 12-24 months of MPH exposure, DAT aAbs titers in PT subjects were comparable to those of healthy controls, while titers remained significantly elevated in NPT patients. Data warrant further research on serum DAT aAbs, which could be used to confirm ADHD diagnosis and/or to monitor therapeutic efficacy of MPH.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) has been internationally recognized as a medical neuro-developmental condition (Curatolo et al., 2009; Davis et al., 2011; Purper-Ouakil et al., 2011; Cortese, 2012); global interest in long-term consequences of ADHD and of psycho-stimulant administration for ADHD is on the rise (Hinshaw et al., 2011). According to the current criteria of Diagnostic and Statistical Manual of Mental Disorders, 4th edition — Text Revision (DSM IV-TR), ADHD prevalent symptoms include problems in maintaining attention, excessive motor activity, and impulsivity, which often lead to poor academic performance and impaired social interactions (American Psychiatric Association, 2000). These symptoms develop early in up to 5% of children (Polanczyk et al., 2007), and can persist into adolescence and adulthood (Biderman et al., 2006). Frequently comorbid with ADHD are other impulse-control disorders, like oppositional defiant disorder, conduct disorder, substance abuse and/or dependence problems (see Hollander et al., 2000, 2005 for pathological gambling), all of which may be conceptualized as part of the addictive disorder spectrum (Fontenelle et al., 2011).

Although the multi-factorial etiology of ADHD is still unclear, evidence suggests that the disorder is linked to imbalanced levels of dopamine neurotransmitter. Some accounts present ADHD as a motivational dysfunction (Sonuga-Barke, 2005), arising from altered processes within fronto-striatal circuits (Oades, 1998; Sagvolden and Sergeant, 1998; Chambers and Potenza, 2003). For this reason, one focus of ADHD research has converged on the brain dopamine transporter (DAT) in the clinics and in preclinical models. It has been proposed that specific ADHD symptoms may arise from a modification in DAT expression and function (Jucaite et al., 2005; Bannon, 2005; Berridge et al., 2007):

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human DAT1 gene has a VNTR polymorphism of 40 bp (3–11 repeats) in the 3′ untranslated region, with 9- and 10-repeat variants being the most common in Caucasians. 10-repeat VNTR polymorphism of DAT has been associated with ADHD and with obsessive-compulsive disorder (Cook et al., 1995; Gill et al., 1997 Sharp et al., 2009; Scherk et al., 2009; Yang et al., 2007; Greenwood et al., 2013). DAT1 high-risk allele (i.e. the 10-repeat allele) can explain 1%–4% of the overall variance in ADHD symptoms; its relation with hyperactive–impulsive symptoms is stronger and more reliable than that with inattentive symptoms (Waldman et al., 1998).

However, although the presence of DAT1 high-risk alleles may influence the severity of the disorder and may explain why as many as 30% of ADHD children do not respond to psycho-stimulants (Madras et al., 2002), other non-genetic factors are crucial for the onset of ADHD symptoms. To account for a possible DAT alteration, a rising interest exists for auto-immune processes and psycho-immunological interactions (Shulman, 2009; Graus et al., 2010a,b). A breach in blood–brain barrier (BBB) integrity, due to conditions of stress (Kuang et al., 2004) or owing to a traumatic injury to the brain (like e.g. as complication of a difficult delivery, Ankeny and Popovich, 2010), could implicate the draining of CNS antigens to peripheral lymphoid organs, with subsequent auto-immune responses (Diamond et al., 2009; Levin et al., 2010). According to recent literature, anti-neuronal antibodies may target a wide range of CNS proteins, including neuro-receptors (Davies et al., 2007; Graus et al., 2008; Zuliani et al., 2012). Behavioral dysfunction might possibly stem from anti-neuronal auto-antibodies (aAbs) that would presumably compromise neural function (Granstrem et al., 2006). Circulating aAbs against neuro-receptors are reliable biomarkers for Systemic Lupus Erythematosus (Cohen-Solal and Diamond, 2011), intractable seizures (Rogers et al., 1994; Twyman et al., 1995), brain ischemic stroke (Dambinova et al., 2003), Hashimoto's encephalopathy (Chong et al., 2003) and Sydenham's chorea. Other two neurological conditions with a claimed role for aAbs and related neuro-psychiatric symptoms are the Limbic and the NMDAR-Ab encephalitis. While seizures are prominent with GABA-B receptor (GABAB-R) aAbs, there may be psychiatric features with AMPA receptor (AMPA-R) aAbs (Lai et al., 2009; Graus et al., 2010a,b; Lancaster et al., 2010; Zandi et al., 2010) and with NMDA receptor (NMDA-R) aAbs (Dalmau et al., 2011). Many of these patients are children who may initially seek for psychiatric wards for acute anxiety, behavioral change or psychosis. Interestingly, a role for auto-immunity in general and for aAbs in particular has been claimed for Tourette's (Hoekstra and Minderaa, 2005; Martino et al., 2009; Rizzo et al., 2010), for obsessive–compulsive disorder (Teixeira et al., 2014), and for ADHD as well (Passarelli et al., 2013; Hegvik et al., 2014).

Circulating aAbs to CNS antigens can be also detected in animal models (Dambinova et al., 1997, 1998; Vincent et al., 1999; Kowal et al., 2006; Knight et al., 2007; Capone et al., 2008; Colasanti et al., 2009), as well as in opiate-treated mice (Granstrem et al., 2006). In this line, we recently proposed (Adriani et al., 2012) that a DAT altered turnover/degradation ratio may lead to an over-production of neuro-receptor fragments, which might then overcome the BBB and spill into the blood, where they can generate an auto-immune reaction. This auto-immune challenge could in turn lead to an enduring and possibly detectable interference with the dopamine (DA) neurotransmission as well as to DA-related behavioral changes, like ADHD symptoms (i.e. impulsivity and hyperactivity). Thus, purpose of the present study was 1) to ascertain the presence in the blood of circulating auto-antibodies (aAbs) targeting some epitopes of the DAT protein, 2) to measure levels of such DAT aAbs, by means of an ELISA assay, as a function of therapy with or without pharmacological MPH treatment, 3) to correlate these DAT aAbs titers with clinical scores of ADHD symptoms, and 4) to further evaluate the above parameters as a function of the individual DAT genotype. We hypothesized that a more clear account of observed symptoms could be served by taking into consideration the interaction between genetic and auto-immune parameters.

2. Material and methods

2.1. Recruitment of patients and healthy controls

Participants included 61 children. We recruited 48 patients with a formal diagnosis of ADHD, with a female to male ratio of 1:5, referred to Child Psychiatry Unit of Tor Vergata University from April 2010 to March 2012. Two of these recruited ADHD children were dropped out, since they turned out to be non-responders to the recruitment commitments; we also recruited 15 healthy children (handled under the same routine conditions from April 2013 to March 2014, in the same period as the patients' re-sampling, see below) to act as controls. All subjects had a full Scale IQ over 84, as assessed by the Wechsler Intelligence Scale — III edition (Wechsler, 1991). They were evaluated by child neuropsychiatrists who determined the diagnosis of ADHD, according to DSM IV-TR criteria (American Psychiatric Association, 2000); a medical work-up excluded any other neuro-genetic disease or immune disorder, as well as any psychiatric comorbidity (conduct disorder, obsessive–compulsive disorder, Tourette's, depression, bipolar disorder, psychosis), assessed by the Schedule for Affective Disorders and Schizophrenia for School Age Children — Present and Lifetime Version (K-SADS-PL) (Kaufman et al., 1997). Post-recruitment exclusions were also made for coeliac or diabetic disease, and in case of recent fever or allergy, as resulting from standard blood screen.

The clinical sample was divided into two, based on therapeutic intervention decided at enrollment: children with milder symptoms, which did not need pharmacological treatment, underwent cognitive-behavioral therapy and/or periodic follow-up (unmedicated, NPT group; $N = 32$); severe children, with a significant impairment of adaptive functioning in different areas of life, were assigned to pharmacological treatment with MPH (PT group; $N = 14$).

The study was formally approved by ISS Ethical Committee (Prot. CE-ISS 09/270 of 15 July 2009, scientific responsible and PI: W.A.). Informed consent procedures included searching for consent from the child (using age-adequate approaches) and illustrating to parents the standard consent form; the parents gave their written informed consent for the child to participate in this study. All potential participants who decided not to participate in the study were not disadvantaged in any way by not participating. Also, we declare that collected biological materials were used solely to the purpose of this study; the responsible person for pediatric privacy is one of authors (M.C.P.). The rules set by the Code of Ethics of the World Medical Association (Declaration of Helsinki), which has been printed in the British Medical Journal (18 July 1964), were respected.

2.2. Clinical assessment

Each patient was evaluated by a staff of trained child neuro-psychiatrists at our "S. Alessandro" clinical Unit, according to the DSM-IV and ICD-10 criteria for ADHD. Information was gathered from the clinical interviews and questionnaires with the parents, teacher and from direct observations of the patients.

Parents completed *SNAP-IV* that elicits DSM-IV TR criteria for ADHD on a four-point scale of frequency (Swanson, 1983), also giving information about ADHD subtypes (inattentive, hyperactive–impulsive, combined type). ADHD symptoms were also determined using *Conners' Parent Rating Scale*; each item was scored according to the published measure from 0 (Not true at all) to 3 (Very much true) (Conners et al., 1998). The semi-structured *Schedule for Affective Disorders and Schizophrenia — Present and Lifetime version* (K-SADS/PL) and also the *Child Behavior Checklist/4–18* (CBCL; Achenbach, 1991) were used separately, to elicit parents' and patients' reports of signs and symptoms that might indicate possible co-morbidities.

The *Children's Global Assessment Scale* (CGAS) was used by clinicians to measure the overall severity of social and psychiatric functioning for children ages of 4–16 years (Shaffer et al., 1983). CGAS scores range between 1 and 100, with higher scores indicating better functioning.