Journal of Neuroimmunology 236 (2011) 72–75

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Twin study on transplacental-acquired antibodies and attention deficit/hyperactivity disorder — A pilot study

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ARTICLE INFO

Article history:
Received 8 December 2010
Received in revised form 10 April 2011
Accepted 24 April 2011

Keywords:
ADHD
Etiology
Infectious Agents
Twins

ABSTRACT

Objective. We hypothesize that maternal transplacentally acquired antibodies may cause Attention Deficit/Hyperactivity Disorder (ADHD) symptoms years after birth, and tested the hypothesis in twins discordant for ADHD symptoms.
Method. In a pre-screened sample of 7793 same sex twin pair's (4–18 years) questionnaire data on hyperactivity and inattention was collected. Blood samples taken 5 days after birth from 190 ADHD-score discordant pairs (15% MZ) were analyzed for antibodies.
Results. Pneumococcus Polysaccaride 14 (PnPs14) was present in the ADHD high scoring twin more often than in the lower scoring twin ($P = 0.04$).
Conclusion. Although the study provides no strong support for the hypothesis, infection or immunological factors may be one among several causes of ADHD. The genetic control obtained in a twin design may reduce the exposure contrast and a larger sample is needed to further explore the role of PnPs14 in the etiology of ADHD.

1. Introduction

Attention Deficit/Hyperactivity Disorder (ADHD) (American Psychiatric Association 1994) is one of the most common psychiatric disorders in childhood and adolescence. It is defined by abnormal behavior in three areas: inattention, hyperactivity and impulsivity. The prevalence in school-aged children is estimated to be between 3% and 6% depending on diagnostic classification system, level of impairment and screening/assessment methodology (Faraone et al. 2003). Despite substantial research the causes of ADHD are not yet known although there are many suspected candidates, such as genes involved in the dopamine hypothesis (Swanson et al. 2007), smoking during pregnancy (Obel et al. 2009) and premature birth. Twin studies indicate that genetic factors and/or prenatal environmental exposure play an important role (Rietveld et al. 2004). Neurodevelopmental disorders like Autism Spectrum Disorder (ASD) and ADHD are probably caused by a combination of genetic and environmental factors (van Loo and Martens, 2007). The fact that ADHD is increasingly being diagnosed is hardly due to sudden changes in the genome. More likely the growing incidence is a result of revised diagnostic criteria and altered clinical practice; but epigenetic or non-genetic changes, perhaps induced by environmental factors like infections may also be involved.

It has been suggested that transplacentally-acquired maternal antibodies to agents with epitope molecular mimicry with the developing nervous system can cross the fetus/infant's blood brain barriers and cause neuro-developmental disorders that become clinically manifest years later (Nahmias et al. 2006). Maternal antibodies have direct access to fetal brain during the first trimester of gestation, and can affect fetal brain development, but the molecular pathways that are triggered or blocked by presumed pathogenic brain-reactive antibodies are not well understood (Diamond et al. 2009). Prematurity has been associated with ADHD and the more immature twin fetus in a pair may be in an increased risk of neural affection by neurotoxins.

Antibody titre levels in newborn babies' plasma can be extracted from frozen dried blood spot taken a few days after birth. If a studied antibody plays a role in altering neural development one would expect the antibody to be more frequently present in the affected individuals than in the unaffected. Maternal infections prenatally or perinatally with clinical manifestations in the offspring have been demonstrated for congenital syphilis and rubella (Webster 1998). It has also been hypothesized that schizophrenia may be caused by infection in prenatal life with significantly elevated IgG titres to toxoplasmosis (Brown et al. 2005;Buka et al. 2001) and influenza (Brown et al. 2004). Brain-reactive antibodies have recently been demonstrated in mothers of patients with autism (Braunschweig et al. 2008;Singer et al. 2008).

In this study we evaluate if specific transplacental-acquired antibodies are a possible cause of ADHD symptoms. A twin design

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doi:10.1016/j.jneuroim.2011.04.012

was selected in which the genetic predisposition to ADHD is controlled. Different antibody load, especially across discordant monozygotic twin pairs, may in this design account for a probable etiological factor.

2. Materials and methods

2.1. Materials

All twins born in Denmark are registered in the Danish Twin Registry (Knudsen and Olsen 1998;Skytthe et al. 2006). The Civil Registration System and the Medical Birth Registry has provided a complete registration of twins born since 1973. This study is based on the birth cohorts 1988–2000. In the first phase of the study parents of 7,793 same-sexed twin pairs participated in a survey including a 5-item behavioral screening questionnaire. The five questions derived from the Child Behavior Checklist (CBCL) (Achenbach 2001) are all scored on a Likert scale (0 = not true, 1 = sometimes true and 2 = often true); range sum 0–10 (Table 1). The CBCL has been validated in Danish populations (Bilenberg 1999;Bilenberg et al. 2005) and the five questions have a high sensitivity for neurodevelopmental disorders; ASD (a,b) and ADHD (d,e) (Nordenbeck et al. in press; Petersen et al. 2006). The questionnaire was returned by 5443 pairs (69.8%). We found 818 same sex pairs in which at least one of the twins reached a score ≥ 3 points in the first survey (Fig. 1).

In the second phase a 5-item ADHD-problem scale (range 0–10) from the Strengths and Difficulties Questionnaire (SDQ) (Goodman et al. 2000) (Table 1) was mailed to the parents of 793 out of the 818 screening positive twin pairs' parents who had agreed to participate. The response-rate was 80.3% leading to data from 637 pairs (23.2% MZ). A twin was classified as having considerable attention-, hyperactivity- and impulsivity problems if rated five or higher on this SDQ subscale. If, at the same time, there was a difference of 4 or more points between the two twins in a pair they were defined as discordant and selected for further analyses. All discordant MZ-pairs (n = 28) and all discordant same-sex DZ-pairs (n = 162) were included in the study.

2.2. Immunological analyses

Filter paper blood samples taken about five days after birth were pulled from freezers for the included pairs and patches of three millimeter of the filter paper were submitted for analysis at Centre for Disease Control and Prevention in Atlanta. Testing and scoring of antibody titres was done in random order and blinded to the ADHD status of the twins. A multiplexed sandwich immunoassay based on flowmetric Luminex® xMAP technology (Earley et al. 2002; Skogstrand et al. 2005) allowed testing of a number of analytes in serum extracted from the blood spots. Assays were performed to assess a battery of antigens determined by what was possible from the limited amount of blood and the technology of the lab. This included IgG antibody binding to microspheres coated with antigens from five infectious agents: *Pneumococcus* polysaccharide serotypes 14 (PnPs14) and 18 (PnPs18)), *Cytomegalovirus* (CMV), *Toxoplasma*, *Herpes simplex viruses* HSV-1 (gG1), HSV-2 (gG2), and a type-common antigen (gD1); two *thyroid autoantigens*: TPO and thyroglobulin; two *heat shock proteins*: HSP70 and HSP90; *polysialic acid* (PSA), six *gangliosides*: GM1, GM2, GD1a, GD1b, GQ1b, GT1a; and five *neural proteins*: brain-derived neural growth factor, glial fibrillar acid protein, myelin basic protein, and medium and light neurofilament proteins. To measure IgG antibodies to these antigens, dried blood spot eluants were first incubated with pooled antigen-coated microspheres and then, after washing, with a fluorescent-labeled goat-anti-human IgG antibody. IgG binding values for each population of antigen-coated microspheres was expressed as the median Relative Fluorescence Unit (RFU) on a scale from 0 to 32,768.

Based on the percentage distribution of RFU values for each analyte, semi-quantitative RFU-scores from 0 to 4 were generated, except for 2–3% with inconclusive values. RFU-scores were then compared within ADHD-discordant twin-pairs. Mean RFU-score difference (ADHD high scoring twin − ADHD low scoring twin) and 95% confidence intervals (CI) were analyzed for all 24 analytes stratified for zygosity and gender. If infection increases the risk of ADHD according to the hypothesis the difference would be > 0. Pearson correlations were made for RFU-scores and ADHD-scale score. All analyses were made in SPSS. [17.0]. (2010).

3. Results

After two screen phases of the 7793 same sex twin pairs (Fig. 1) we had 190 pairs (2.4%) with discordant ADHD-score; one twin scoring ≥ 5 points and the co-twin scoring 4 or more points below. For 27 out of 28 discordant MZ pairs (19 male-pairs, 8 female-pairs) with a mean age of 9.9 years; and for 139 out of 162 same-sex discordant DZ twin pairs (87 male-pairs and 52 female-pairs) with mean age of 8.7 years, dried blood spots were available for analysis (Fig. 1 and Table 2).

In 55% of the 166 pairs both twins had exactly the same antigen/protein level (RFU-score) for all 24 analytes. Mean RFU-score differences between ADHD high scoring and low scoring twins were close to 0 for most agents. The only significant difference was for the PnPs14 titre. Within 16 twin-pairs (11 DZ and 5 MZ) the potential ADHD twin had higher PnPs14 RFU-score than the low ADHD scoring co-twin, whereas it was opposite in only 4 pairs (all DZ). The mean difference was 0.08 (0.01;0.15). For MZ twin pairs (n = 26) the mean difference was 0.19 (0.03;0.35). Using Wilcoxon Signed Rank Test the *P*-value was 0.04. RFU-score were also higher for CMV antibody in affected twin girls (n = 48), where the mean difference was 0.17 (0.03;0.31).

Mean birth weights (BW) for the ADHD high scoring twins was 30 grams lower than that of the lower scoring (see Table 2) but the difference was not significant, neither in the MZ nor DZ pairs (paired *T*-test). For low BW twins (one or both below 1000 g) it was often the twin with the lowest BW who was the "ADHD case" (in 4 out of 5 pairs) (n.s.). BW within twin pairs differed by more than 500 grams in 50 pairs, and in 30 pairs the "ADHD case" was the one with the lower BW (n.s.).

Table 1
The questions used in the two stage design to identify the Attention Deficit/Hyperactivity Disorder (ADHD) symptoms in twins.

CBCL[a] items used in screening	SDQ[b] ADHD-items used in the second stage
The child exhibits strange behavior	Restless, overactive, cannot stay still for long
The child exhibits a sudden change in mood or feelings	Constantly fidgeting or squirming
The child can't get along with others	Easily distracted, concentration wanders
The child can't sit still, is restless or hyperactive	Thinks things out before acting[c]
The child can't pay attention for long	Sees tasks through to the end, good attention span *

[a] Child Behavior Checklist (CBCL); Total score range 0–10. Cut off used in the first stage was one or both twins in a pair scored ≥ 3 points.
[b] Strength and difficulties questionnaire (SDQ); ADHD "case" was defined as a twin scoring ≥ 5. Discordancy was defined as a difference between twins of ≥ 4.
[c] The items are positively framed and rated reversely.