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Decreased serum levels of adiponectin in adult attention deficit hyperactivity disorder

Thegna Mavroconstanti [a,b,*], Anne Halmøy [a,b], Jan Haavik [a,b]

[a] K.G. Jebsen Centre for Research on Neuropsychiatric Disorders, Department of Biomedicine, University of Bergen, 5009 Bergen, Norway
[b] Division of Psychiatry, Haukeland University Hospital, Bergen, Norway

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ABSTRACT

The main aim of this study was to investigate serum levels of adiponectin in adult patients with attention deficit hyperactivity disorder (ADHD). The second objective was to examine the effects of rare missense mutations in T-cadherin, an adiponectin receptor encoded by the ADHD candidate gene *CDH13*, on serum adiponectin levels. Total and high molecular weight (HMW) adiponectin levels were measured by an enzyme-linked immunosorbent assay in 44 patients and 29 controls. We found decreased serum adiponectin levels in ADHD patients. In a logistic regression model, adjusting for confounding by age, body mass index, and gender, HMW adiponectin and its ratio to total adiponectin were significantly associated with ADHD. In partial correlations, HMW adiponectin and its ratio to total adiponectin were significantly inversely correlated with self-reported psychiatric symptomatology. A non significant trend for higher levels of total adiponectin was observed in patients carrying *CDH13* missense mutations compared to patients with wild type *CDH13*. The association of *CDH13* mutations with adiponectin levels should be investigated in larger studies. This study shows that ADHD patients have decreased serum adiponectin levels, which are inversely correlated to psychiatric symptoms, suggesting a possible involvement of adiponectin, in particular the HMW form, in the pathophysiology of ADHD.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a common childhood neurodevelopmental disorder with worldwide prevalence estimates of around 5–10% (Faraone et al., 2003; Polanczyk et al., 2007). ADHD often persists into adulthood with a prevalence of around 3–5% in young adults (Fayyad et al., 2007; Simon et al., 2009). Although around 75% of the variability in childhood ADHD symptomatology is accounted for by genetic factors (Faraone and Doyle, 2001), unequivocal genetic associations with ADHD have not been identified yet (Faraone et al., 2005; Franke et al., 2012). Research in ADHD etiology has revealed several biological and psychosocial risk factors for this disorder, such as maternal smoking (Langley et al., 2005) and alcohol consumption during pregnancy (Banerjee et al., 2007), pre-term birth and low birth weight (Halmoy et al., 2012), maternal stress, environmental toxin exposure, and childhood adversity (Biederman, 2005). Still, the biological mechanisms mediating these risk factors have not yet been identified and few biomarkers have shown consistent associations with ADHD (Scassellati et al., 2012).

An increased prevalence of obesity, cardiovascular disease and diabetes mellitus has been reported in disorders like major depression, bipolar disorder, and schizophrenia (Bai et al., 2013; Stanley and Laugharne, 2012; Stanley et al., 2013). Likewise, recent studies have shown co-occurrence of obesity and ADHD in children (Agranat-Meged et al., 2005; Halfon et al., 2013) and adults (Fleming et al., 2005). Moreover, there is evidence that obesity genes such as the *FTO* gene, which codes for the enzyme alpha-ketoglutarate-dependent dioxygenase, may affect ADHD risk (Choudhry et al., 2013) and that obesity and ADHD may share common risk alleles (Albayrak et al., 2013). Thus, these co-morbidities may reflect a common etiology or the involvement of common pathways, as well as a cross-talk between adipose tissue and the central nervous system (Schulz et al., 2010).

In line with these findings, abnormal circulating levels of hormones secreted by adipose tissue, such as the adipocytokine adiponectin, have been detected in obesity (Arita et al., 1999; Ryo et al., 2004), type II diabetes and insulin resistance (Kadowaki et al., 2006; Yatagai et al., 2003), but also in patients with psychiatric disorders such as major depression (Leo et al., 2006), schizophrenia (Cohn et al., 2006), panic disorder (Unsal et al., 2012) and bipolar disorder (Barbosa et al., 2012). Adiponectin is an

* Corresponding author at: K.G. Jebsen Centre for Research on Neuropsychiatric Disorders, Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway.
E-mail addresses: tma079@biomed.uib.no, tma079@gmail.com (T. Mavroconstanti).

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adipokine hormone that has insulin-sensitizing (Shehzad et al., 2012) and anti-inflammatory effects (Wolf et al., 2004), stimulates fatty acid oxidation (Yamauchi et al., 2001), and its expression is regulated by insulin (Scherer et al., 1995), testosterone (Nishizawa et al., 2002), and glucocorticoids (Sukumaran et al., 2012). Adiponectin molecules circulate in the blood mainly as trimers of 30 kDa subunits and multimers composed of combinations of trimers and hexamers (Kadowaki and Yamauchi, 2005; Tsao et al., 2003). The diverse functions of adiponectin are mediated by the AdipoR1 and AdipoR2 receptors that show predominant expression in muscle and liver, respectively, (Yamauchi et al., 2003) but are also expressed in the brain (Thundyil et al., 2012). A third adiponectin receptor, T-cadherin, selectively binds the hexameric and high molecular weight forms (HMW) of adiponectin and is abundantly expressed in the cardiovascular system and the brain (Hug et al., 2004). Several genome wide association (GWA) studies have detected associations between single nucleotide polymorphisms (SNPs) in the region of the *CDH13* gene, which codes for T-cadherin, and ADHD (Lasky-Su et al., 2008; Lesch et al., 2008). Moreover GWA studies have shown a strong association of *CDH13* polymorphisms with serum levels of adiponectin (Morisaki et al., 2012; Wu et al., 2010). Based on these findings, we wanted to examine serum adiponectin levels in ADHD and the effects of missense mutations in *CDH13* on serum adiponectin levels.

The main aim of this study was to compare serum adiponectin levels, and adiponectin multimer distribution, in a sample of ADHD patients and population derived controls. The second aim was to investigate the effects of missense heterozygous *CDH13* mutations, previously identified in our sample (Mavroconstanti et al., 2013) on the serum levels of adiponectin by comparing two subgroups of adult ADHD patients: (1) carriers of wild type *CDH13* and (2) carriers of either one of seven rare *CDH13* mutations.

2. Methods

2.1. Subjects and measures

The present study is part of a large multidisciplinary study of ADHD at the University of Bergen, Norway. Most of the patients in the study were recruited from a national registry of adults (> 18 years) diagnosed with ADHD between 1997 and 2005. Additionally, adult patients diagnosed after 2005 were recruited from outpatients clinics nationwide (Halmoy et al., 2009). The inclusion criteria was a formal diagnosis of ADHD or hyperkinetic disorder made by a clinician (psychiatrist or psychologist) according to ICD-10 or DSM-IV criteria, before entering the study (Johansson et al., 2008). The Medical Birth Registry of Norway was used to randomly recruit controls from the general population in the same range of age (Halmoy et al., 2009). There were no formal exclusion criteria. Blood was obtained between 9 am–4 pm and serum was subsequently collected and stored at −80 °C. All the study participants completed self-report questionnaires with a total of 110 questions relating to different comorbidities, psychiatric symptoms and treatment history, including (1) the adult ADHD self-report scale (ASRS) rating current symptoms of ADHD (Kessler et al., 2005), (2) the Wender Utah rating scale (WURS) rating retrospectively reported ADHD related symptoms in childhood (Ward et al., 1993), (3) the cyclothymic subscale of the temperament evaluation of Memphis, Pisa, Paris and San-Diego (TEMPS-A) (Akiskal et al., 2005) and (4) and the Mood Disorder questionnaire (MDQ)(Hirschfeld et al., 2000), a screening questionnaire for bipolar disorder. The ASRS consists of 18 items corresponding to DSM-IV criteria for ADHD, the first 9 assessing symptoms of inattention (ASRS In), the last 9 symptoms of hyperactivity/impulsivity (ASRS Hyp/Imp). A more detailed description of the methodology used to recruit patients and controls, and also the questionnaires used in the study, can be found in a previous publication (Halmoy et al., 2009, 2010; Landaas et al., 2012).

The total sample in the current study ($n=73$) was comprised of 44 adult ADHD patients and 29 controls. The controls were selected based on blood sample availability whereas patient selection was additionally based on the availability of *CDH13* genotype information. The patient sample consisted of 27 randomly selected carriers of wild type *CDH13* and 17 heterozygous carriers of either one of seven rare coding mutations in the *CDH13* gene (V112I (rs200199969), G113R (rs183971768), R174W (novel), A376T (rs35549391), I585V (rs199759196), L643R (rs34106627), and N39S (rs72807847)) previously detected in a sample of 641 adult ADHD patients with a combined allele frequency of 3.24% (Mavroconstanti et al., 2013). The effects of *CDH13* mutations on adiponectin levels were investigated in the subgroups of patients carrying wild type or mutant CDH13.

2.2. Ethics statement

The study was approved by the Norwegian Regional Medical Research Ethics Committee West (IRB #3 FWA00009490, IRB00001872) and conducted according to the principles of the declaration of Helsinki (2008). All participants signed a written informed consent form.

2.3. Serum total and HMW adiponectin measurements

The measurements of total and HMW adiponectin levels in the serum obtained from 44 patients and 29 healthy controls were performed using a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Quantikine ELISA Human Total and HMW Adiponectin/Acrp30 Immunoassays, R&D systems). For the measurements, serum samples were centrifuged at 1000*g* for 10 min and diluted 100-fold. According to the manufacturer (R&D systems), the sensitivity (mean minimum detectable dose) is 0.246 ng/ml for total adiponectin and 0.195 ng/ml for HMW adiponectin. The intra-assay precision (coefficient of variation; CV) was specified to be 2.5–4.7% for total adiponectin and 2.6–3.7% for HMW adiponectin. The inter-assay precision was specified to be 5.8–6.9% for total adiponectin and 8.3–8.6% for HMW adiponectin. All assays were performed in duplicate. Ten random samples were subjected to four repeated measurements over a 6 month period. The observed intra-assay CV varied between 2.2% and 3.8% for total adiponectin, 0.8–3.7% for HMW adiponectin and the inter-assay CV varied between 7.5% and 17.0% for total adiponectin and 6.3–18.4% for HMW adiponectin.

2.4. Statistical analyses

IBM SPSS version 19 (SPSS Inc., Chicago, Illinois) was used for the statistical analyses. Differences in adiponectin levels, as well as other differences of continuous variables between groups, were analyzed using either a *t*-test for normally distributed variables or a nonparametric Mann Whitney test for variables with skewed distributions. The distribution of each variable in the total sample was examined by both the Kolmogorov–Smirnov and the Shapiro–Wilk normality tests. Pearson's chi-squared exact test was used to analyze differences of categorical variables between groups. To study correlations between adiponectin levels and psychiatric symptoms, based on self-report questionnaire scores (WURS, ASRS, TEMPS-A, MDQ), we performed partial correlations for controlling the effects of body mass index (BMI) and age in the total sample of patients and controls.

To study the associations between adiponectin levels and (1) an ADHD diagnosis, or (2) the subcategories of patients carrying wild type or mutant CDH13 we used logistic regression models (method enter). Statistically significant results were adjusted for the effects of BMI, age and gender. To examine the association between adiponectin levels and an ADHD diagnosis, we defined the binary categorical variable ADHD (yes/no) as the outcome variable and the levels of total, HMW, or the percentage of HMW to total adiponectin as the predictor variables. Total, HMW or the percentage of HMW/total adiponectin was entered individually in step one of the model to observe the unadjusted associations of each variable with an ADHD diagnosis. Statistically significant results were subsequently adjusted for the effects of age, gender and BMI which were added in step 2, both individually and as a group. In an analogous logistic regression model we examined the association of adiponectin levels with the binary outcome variable of the subcategories of patients carrying wild type or mutant *CDH13*. Moreover, to investigate possible effects of ADHD co-morbid disorders or medication use on adiponectin levels, we performed logistic regression analyses stratified for each comorbid disorder or medication use, in patients only.

3. Results

3.1. Sociodemographic and clinical characteristics of the study participants

The sociodemographic and clinical characteristics of (1) the ADHD patient and control groups and (2) the subgroups of ADHD patients who were carriers of wild type or mutant *CDH13* are presented in Tables 1 and 2, respectively. No statistically significant differences were observed for the distribution of gender, age and BMI between ADHD patients and controls. Compared to controls, ADHD patients had significantly lower levels of total ($P=0.001$) and HMW ($P<0.001$) adiponectin as well as a lower