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Inter-hemispherical asymmetry in default-mode functional connectivity and *BAIAP2* gene are associated with anger expression in ADHD adults

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ABSTRACT

Attention deficit hyperactivity disorder (ADHD) is accompanied by resting-state alterations, including abnormal activity, connectivity and asymmetry of the default-mode network (DMN). Concurrently, recent studies suggested a link between ADHD and the presence of polymorphisms within the gene *BAIAP2* (i.e., brain-specific angiogenesis inhibitor 1-associated protein 2), known to be differentially expressed in brain hemispheres. The clinical and neuroimaging correlates of this polymorphism are still unknown. We investigated the association between *BAIAP2* polymorphisms and DMN functional connectivity (FC) asymmetry as well as behavioral measures in ADHD adults. Resting-state fMRI was acquired from 30 ADHD and 15 healthy adults. For each subject, rs7210438 and rs8079626 within the gene *BAIAP2* were genotyped. ADHD severity, impulsiveness and anger were assessed for the ADHD group. Using multivariate analysis of variance, we found that genetic features do have an impact on DMN FC asymmetry. In particular, polymorphism rs8079626 affects medial frontal gyrus and inferior parietal lobule connectivity asymmetry, lower for AA than AG/GG carriers. Further, when combining FC asymmetry and the presence of the rs8079626 variant, we successfully predicted increased externalization of anger in ADHD. In conclusion, a complex interplay between genetic vulnerability and inter-hemispherical DMN FC asymmetry plays a role in emotion regulation in adult ADHD.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is characterized by marked impulsiveness and attentional deficits. It has a prevalence of 5–8% in childhood (Bush, 2010), and persists in adulthood in 60% of the cases with an established prevalence of 2.5–4.9%, (Simon et al., 2009). Depending on the presence and severity of ADHD cardinal symptoms (i.e., hyperactivity, impulsiveness and inattention), predominantly hyperactive/impulsive, inattentive and combined subtypes have been described (Biederman and Faraone, 2006; Fried et al., 2006; Babinski et al., 2011; Doshi et al., 2012; Chang et al., 2014; Ginsberg et al., 2014). Moreover, the poor regulation of emotions defines an additional dimension in ADHD characterized by a difficulty to control anger and tolerate frustration (Shaw et al., 2014). This latter dimension has undeniable consequences on the global functioning, quality of life, professional and social achievements, and interpersonal relationships of ADHD patients (Marx et al., 2011).

Etiologically, both environmental and genetic factors have been implicated in ADHD with an heritability estimated between 60% and 90% (Stergiakouli and Thapar, 2010). Several genes encoding neurotrophic factors and their receptors have been associated with ADHD (Ribases et al., 2008). In addition, previous lines of evidence supported a relationship between neurodevelopmental genes, characterized by an asymmetric expression in brain hemispheres, and vulnerability to ADHD (Ribases et al., 2009). Among these, adult ADHD was significantly associated with a haplotype constituted of two single

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nucleotide polymorphism markers, rs7210438 and rs8079626 (Ribases et al., 2009), located in *BAIAP2* (brain-specific angiogenesis inhibitor 1-associated protein 2), a gene known to be involved in neuronal proliferation, survival and maturation during early development (Knusel et al., 1990; Beck et al., 1993; Russo et al., 2007).

From a neuroimaging viewpoint, compelling evidence points to rather large-scale abnormalities in network organization in ADHD (Sergeant et al., 2006; Konrad and Eickhoff, 2010; Cao et al., 2013), affecting both functional (Cocchi et al., 2012; Colby et al., 2012; Fair et al., 2012; Tomasi and Volkow, 2012; Cao et al., 2013; Di Martino et al., 2013) and structural (Cao et al., 2013; Hong et al., 2014) connectivity. Moreover, an abnormal hemispheric asymmetry of brain structure and function was also consistently reported in ADHD (Dennis and Thompson, 2013; Shang et al., 2013; Cao et al., 2014; Hale et al., 2014, 2015; Keune et al., 2015; Silk et al., 2015), suggesting a possible neurodevelopmental scenario for this disorder.

In terms of neural networks, whole brain resting-state functional imaging studies have reported abnormalities in the well-known Default Mode Network (DMN) (Sonuga-Barke and Castellanos, 2007; Castellanos et al., 2008; Fair et al., 2010; Tomasi and Volkow, 2012; Di Martino et al., 2013; Hale et al., 2014), a “task-negative” network including cortical areas that show temporally-coherent activity during the resting condition (i.e., posterior cingulate cortex (PCC), retrosplenial cortex, inferior parietal lobule, lateral temporal cortex, medial prefrontal cortex and hippocampal formation) (Buckner et al., 2008). These abnormalities included a decreased DMN functional activation (Hale et al., 2014), a delayed DMN maturation (Fair et al., 2010) and a structural/functional right-biased DMN asymmetry (Hale et al., 2014). Interestingly, a default-mode interference hypothesis in ADHD was firstly introduced by Sonuga-Barke and Castellanos (Sonuga-Barke and Castellanos, 2007), who postulated that the DMN fails to decrease its activity when switching to an active task in ADHD. In normal conditions, a fronto-parietal “task-positive” network (TPN), including dorsolateral prefrontal cortex, intraparietal sulcus, and supplementary motor area, antagonizes the DMN and is strongly activated during complex attentional tasks. Imbalances in the interplay between DMN and TPN have been thought to be at the origin of attentional deficits in ADHD (Castellanos et al., 2009). In terms of connectivity, an impairment between frontal brain regions and posterior DMN (precuneus and PCC) was observed (Castellanos et al., 2008) and converging results from voxel-based morphometry showed that decreased volume of the posterior DMN areas correlates with altered DMN connectivity (Castellanos et al., 2009).

The contribution of genetic factors in ADHD-related DMN dysfunction - in particular inter-hemispheric asymmetry - as well as their relevance in respect to clinical ADHD patterns are still poorly understood. To this purpose, the present work proposes a multivariate approach combining neuroimaging, genetics and behavioral information, to further elucidate the pathological mechanisms of ADHD (Dennis and Thompson, 2013). Using a cross-sectional design, we investigated the interactions between two polymorphisms within *BAIAP2* (rs7210438 and rs8079626) and DMN inter-hemispheric asymmetry in a sample of adult ADHD patients compared to controls. We also explored possible associations between the *BAIAP2* polymorphisms, DMN inter-hemispheric asymmetry and clinical parameters (overall disease severity, anger and impulsiveness) in our cohort.

2. Methods

2.1. Subjects

Thirty right handed adult ADHD patients (21 men; mean age ± SD = 38.7 ± 9.9 years) were recruited in a specialized program for adult with ADHD (Table 1). Diagnosis was made according to the Diagnostic and Statistical Manual (DSM) IV-TR criteria, based on clinical assessment by trained psychiatrists (NP and BP) in addition to using the French version of the Diagnostic Interview for Genetic Studies (DIGS) - a semi-structured interview including a detailed investigation of childhood ADHD and its persistence into adulthood (Preisig et al., 1999). The diagnosis was confirmed for all of the cases by a best estimate procedure. All subjects also filled out the Adult Self-Report Scale (ASRS-1.1) (Adler et al., 2006) exploring current ADHD symptoms, the Barrat Impulsiveness Scale (BIS-10) (Patton et al., 1995) investigating impulsivity (dimensions: attentional motor and non-planning impulsiveness), and the State-Trait Anger Expression Inventory (STAXI) (Spielberger, 1988), measuring the following anger dimensions: anger out, anger in, anger control, state anger and trait anger. All patients treated with methylphenidate stopped their medication 48 h before the fMRI recording.

A group of fifteen right handed healthy controls (4 men; mean age ± SD = 32.2 ± 5.5 years) was also recruited and screened for head trauma, neurological disorders, and crucially for current/past psychiatric disorders as well as subjective complaints of ADHD. Every participant was required to fill out the Adult ASRS-1.1 (Adler et al., 2006). All subjects were from European ancestry for at least two generations. The study was approved by the ethics committee of the University Hospitals of Geneva, Switzerland, and all subjects gave their informed written consent.

2.2. Genotyping

2.2.1. BAIAP2 rs8079626

Genomic DNA was extracted from peripheral blood using the Nucleon™BACC3Kit (Amersham plc, Buckingham shire, UK). Qualities of DNA were estimated by agarose gel electrophoresis and DNA was quantified with Maestro Nano Spectrophotometer (Maestrogen, LasVegas, NV, USA).

Genotyping was identified by high-resolution melt (HRM) assay on a Rotor-Gene 6000 instrument (Corbett Life Science, Australia). In each HRM assay, we used three controls as standards of each possible genotype determined by capillary sequencing at the Genomic platform of Geneva university after PCR amplification using the following conditions: a 370 bp PCR product was obtained by PCR amplification on DNA samples using forward primer 5′- TTTGGCTGTTGTTGTGTGTG-3’ and reverse primer 5′-GTGCAGCAGGCAGAATACAA-3′. PCR reactions were performed in 25 µl final volume containing 100 ng of DNA, 1× ThermoPOL Reaction Buffer (New England Biolabs, cat.num: M0267L), 1.6Mm MgCl2 (New England Biolabs, cat.num: B9021S), 200 µM Dntp (New England Biolabs, cat.num: N0447L), 0.20 Mm of each Forward and Reverse primers, 2 units of HotStart Taq DNA polymerase (Biolabs, cat.num: M0267L). PCR amplification were perform ed as follows: 95 °C during 3 m in, 30 cycles of 95 °C during 30 s, 58 °C during 30 s and 72 °C during 30 s.

PCR and HRM conditions were as follows: PCR reaction was carried out with 100 ng of genomic DNA using Kappa 2 G Robust Hot Start Kit (Kappa Biosystem, Cape Town, South Africa) in a final volume of 20 µl containing 1x buffer A, 0.02 mM dNTPs, 7.5 µM of each primer (designed with Primer3 site) 5′- TTGTTGTGTGTGCCTGTTTTT-3’ forward type and 5′-CGTGCCAAGATCAGCAGTT -3′ reverse type, 0.01 mM Hot Start polymerase and 0.04 µM Eva Green fluorescent intercalating dye (Quantace, London, United Kingdom). Amplification conditions were as follows: 95 °C for 3 min, 45 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 20 s. Immediately following PCR cycling, the HRM was set from 68 °C to 90 °C, with the temperature rising by 0.2 °C per second. Each sample was tested in duplicate and each of our experimental runs included the three standards.

2.2.2. BAIAP2 rs7210438

Genotyping was identified by capillary sequencing at the Genomic platform of Geneva university after PCR amplification using the following conditions: a 426 bp PCR product was obtained by PCR amplification on DNA samples using forward primer 5′ -