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Genome-wide copy number variation analysis in adult attention-deficit and hyperactivity disorder

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ABSTRACT

Attention-deficit and hyperactivity disorder (ADHD) is a common psychiatric disorder with a worldwide prevalence of 5–6% in children and 4.4% in adults. Recently, copy number variations (CNVs) have been implicated in different neurodevelopmental disorders such as ADHD. Based on these previous reports that focused on pediatric cohorts, we hypothesize that structural variants may also contribute to adult ADHD and that such genomic variation may be enriched for CNVs previously identified in children with ADHD. To address this issue, we performed for the first time a whole-genome CNV study on 400 adults with ADHD and 526 screened controls. In agreement with recent reports in children with ADHD or in other psychiatric disorders, we identified a significant excess of insertions in ADHD patients compared to controls. The overall rate of CNVs >100 kb was 1.33 times higher in ADHD subjects than in controls (p = 2.4e-03), an observation mainly driven by a higher proportion of small events (from 100 kb to 500 kb; 1.35-fold; p = 1.3e-03). These differences remained significant when we considered CNVs that overlap genes or when structural variants spanning candidate genes for psychiatric disorders were evaluated, with duplications showing the greatest difference (1.41-fold, p = 0.024 and 2.85-fold, p = 8.5e-03, respectively). However, no significant enrichment was detected in our ADHD cohort for childhood ADHD-associated CNVs, CNVs previously identified in at least one ADHD patient or CNVs previously implicated in autism or schizophrenia. In conclusion, our study provides tentative evidence for a higher rate of CNVs in adults with ADHD compared to controls and contributes to the growing list of structural variants potentially involved in the etiology of the disease.

1. Introduction

Attention-deficit and hyperactivity disorder (ADHD) is a common psychiatric disorder with a worldwide prevalence of 5–6% in children and 4.4% in adults that is characterized by pervasive and impairing symptoms of inattention, hyperactivity and impulsivity (Biederman and Faraone, 2005; Kessler et al., 2006; Polanczyk et al., 2007). Family, twin and adoption studies suggest an essential contribution of genetic factors in the etiology of the disorder, with an estimated heritability of 76% (Biederman, 2005; Biederman and Faraone, 2005; Faraone et al., 2005).

Although studies on ADHD have mainly considered children and the heritability estimates of ADHD in adults are substantially lower than in children (around 30%–50%) (Boomsma et al., 2010; Larsson et al., 2013; Kan et al., 2013), converging evidence suggests a stronger genetic component involved in the etiology of adult ADHD (Biederman et al., 1996, 1995; Faraone et al., 2000a, 2000b).

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The research on the genetics underlying this psychiatric disorder has mainly focused on common variants through candidate gene association studies or hypothesis-free genome-wide scans (GWAS) (Faraone et al., 2005; Fliers et al., 2012; Gizer et al., 2009; Hinney et al., 2011; Lantieri et al., 2010; Lasky-Su et al., 2008a, 2008b; Lesch et al., 2012; Mick et al.; Neale et al., 2008, 2010; Poelmans et al., 2011). Recent work, however, strengthens the hypothesis that copy number variations (CNVs) encompassing relatively large genomic regions might contribute to the susceptibility of different psychiatric disorders such as ADHD (Kirov et al., 2008; Malhotra et al., 2011; Schizophrenia, 2008; Stefansson et al., 2008; Walsh et al., 2008; Stephansson et al., 2008; Coe et al., 2012). To date, eight genome-wide association studies focusing on structural variants have been reported in ADHD and showed promising results (Elia et al., 2010, 2011; Jarick et al., 2012; Lesch et al, 2012; Lionel et al., 2011; Stergiakouli et al., 2012; Williams et al., 2012, 2010). All of them considered childhood ADHD patients and only three reported a greater CNV burden in the ADHD dataset when compared to controls (Stergiakouli et al., 2012; Williams et al., 2012, 2010). In this regard, Williams et al. provided evidence supporting enrichment of large, rare CNVs in children with ADHD, mainly due to an excess of duplications at 16p13.11 or 15q13.3 loci (Williams et al., 2012, 2010). Enrichment of rare CNVs affecting genes that belong to the metabotropic glutamate receptor gene family was also reported in multiple ADHD cohorts (Elia et al., 2011), while an excess of deletions and duplications at the *PARK2* locus at 6q25.2-q27 was identified in children with ADHD (Jarick et al., 2012). Although other studies failed to identify a higher rate of CNVs in ADHD patients, they reported enrichment of ADHD-related CNVs at loci previously associated with different neurodevelopmental disorders such as autism and/or schizophrenia and provided further evidence in support of the hypothesis that these disorders may share genetic susceptibility factors (Elia et al., 2010; Lionel et al., 2011; Williams et al., 2012, 2010).

In this line and based on previous reports pointing at common risk variants in both childhood and adult ADHD, we hypothesized that structural variants may contribute to ADHD in adults and that such genomic variation may be enriched for CNVs previously identified in children with ADHD (Faraone et al., 2000c; Kuntsi et al., 2005; Ribases et al., 2009b, 2011). To address this issue, we investigated the role of CNVs in adult ADHD and performed for the first time a genome-wide CNV association study on 400 adults with ADHD and 526 controls in which ADHD symptomatology was discarded. Subsequently, we investigated whether the identified CNVs in our adult cohort with ADHD were significantly enriched for loci previously implicated in childhood ADHD or other neuropsychiatric disorders such as autism or schizophrenia.

2. Materials and methods

2.1. Subjects and clinical assessment

A total sample of 400 ADHD subjects (254 combined, 133 inattentive, 9 hyperactive-impulsive and four with undefined subtype) and 526 sex-matched unrelated controls were recruited from Hospital Vall d'Hebron, Barcelona (Spain). Sixty-seven percent of patients were male ($n = 267$). The average age at assessment was 31.16 years (SD = 12.5) for patients and 33.2 years (SD = 21.5) for controls. The ADHD diagnosis was based on the Structured Clinical Interview for DSM-IV Axis I and Axis II Disorders (SCID-I and SCID-II) and the Conners' Adult ADHD Diagnostic Interview for DSM-IV (CAADID). The level of impairment was measured by the Clinical Global Impression (CGI) included in the CAADID Part II and the Sheehan Disability Inventory. Exclusion criteria for the adult and childhood Spanish patients cohorts were IQ < 70; pervasive developmental disorders; schizophrenia or other psychotic disorders; the presence of mood, anxiety or personality disorders that might explain ADHD symptoms; adoption; sexual or physical abuse; birth weight <1.5 kg; and other neurological or systemic disorders that might explain ADHD symptoms.

All controls consisted of Caucasian blood donors in which DSM-IV life-time ADHD symptomatology was excluded under the following criteria: (1) not having previously been diagnosed with ADHD and (2) answering negatively to the life-time presence of the following DSM-IV ADHD symptoms: (a) often has trouble keeping attention on tasks, (b) often loses things needed for tasks, (c) often fidgets with hands or feet or squirms in seat and (d) often gets up from seat when remaining in seat is expected. Due to ethics concerns, all subjects included as controls were adults.

2.2. CNV calling

Genome-wide genotyping was performed with the Illumina HumanOmni1-Quad platform in 607 adults with ADHD and 584 healthy controls. CNV analysis was limited to 1,109,421 autosomal SNPs. BeadStudio was used to determine the Log R Ratio (LRR) and B allele frequency (BAF) at each SNP according to standard Illumina protocols. CNVs were defined by PennCNV (Wang et al., 2007). A total sample of 400 adult ADHD cases and 526 controls was considered for the analysis after quality control filtering that excluded samples showing high standard deviation in their genome-wide LRR (>0.30), carrying more than 100 CNVs with more than 10 SNPs, carrying more than 30 CNVs larger than 100 kb or showing a |GC base pair wave factor (GCWF)| > 0.04. Adjacent CNV calls were merged together into one single call when the gap between them was less than 20% of the entire length of the new merged CNV. CNVs with more than 50% of their length spanning known segmental duplications (NCBI Build 36.1, hg18), common CNVs defined by the Genome Structural Variation Consortium (http://projects.tcag.ca/variation/ng42m_cnv.php) or known gaps of at least 200 kb in the SNP array were excluded from the study. The statistical analysis included all common and rare (<1% in the overall sample), large CNVs (>100 kb). CNVs were also stratified according to the CNV type (deletion or duplication). When several CNVs identified in different samples overlapped, they were merged in a single locus encompassing all overlapping CNVs, and the number of CNVs within each test region in the group of patients and controls was determined. Significance of the burden comparisons was assessed through permutation one-sided tests (100,000 permutations) using PLINK (version 1.06) (Purcell et al., 2007).

To evaluate whether CNVs identified in our ADHD cohort were significantly enriched for CNV loci previously associated with childhood ADHD or CNVs previously identified in at least one ADHD subject, we first defined from previous reports the genomic coordinates for a list of 15 or 720 independent CNVs, respectively (Elia et al., 2011; Jarick et al., 2012; Williams et al., 2012, 2010; Bradley et al., 2010; Elia et al., 2010; Langley et al., 2011; Lesch et al., 2012; Lionel et al., 2011; Stergiakouli et al., 2012) (Table S1 and S2) and tested the overall significance for the total burden of CNVs at these loci between ADHD cases and controls. The overall CNV content (with and without overlapping genes) was considered to evaluate whether the CNVs identified were enriched with genes related to psychiatric disorders or with CNVs related to ADHD. Pathway analyses were performed using the Ingenuity Pathway Analysis (IPA) software package (http://www.ingenuity.com).

3. Results

400 adult ADHD patients and 526 controls were included in the final study. Fifty-two percent of controls had ≥1 CNV call (6 being