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ORIGINAL PAPER

ADGRL3 rs6551665 as a Common Vulnerability Factor Underlying Attention-Deficit/Hyperactivity Disorder and Autism Spectrum Disorder

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Abstract

Neurodevelopmental disorders are prevalent, frequently occur in comorbidity and share substantial genetic correlation. Previous evidence has suggested a role for the *ADGRL3* gene in Attention-Deficit/Hyperactivity Disorder (ADHD) susceptibility in several samples. Considering ADGRL3 functionality in central nervous system development and its previous association with neurodevelopmental disorders, we aimed to assess *ADGRL3* influence in early-onset ADHD (before 7 years of age) and Autism Spectrum Disorder (ASD). The sample comprises 187 men diagnosed with early-onset ADHD, 135 boys diagnosed with ASD and 468 male blood donors. We tested the association of an *ADGRL3* variant (rs6551665) with both early-onset ADHD and ASD susceptibility. We observed significant associations between *ADGRL3*—rs6551665 on ADHD and ASD susceptibilities; we found that G-carriers were at increased risk of ADHD and ASD, in accordance with previous studies. The overall evidence from the literature, corroborated by our results, suggests that *ADGRL3* might be involved in brain development, and genetic modifications related to it might be part of a shared vulnerability factor associated with the underlying neurobiology of neurodevelopmental disorders such as ADHD and ASD.

Keywords ADHD · ASD · *ADGRL3* · *LPHN3* · Neurodevelopment · Sex-specific effects

Introduction

Neurodevelopmental disorders, as characterized by DSM-5, include a group of neuropsychiatric conditions that generally manifest symptoms before adolescence, follow a stable course throughout adulthood and are more prevalent in males (APA 2013; Thapar et al. 2015). Among these, Attention-Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) are some of the most common and more classical examples of neurodevelopmental disorders (APA 2013). While ADHD is characterized by the presence of attentional problems and a pattern of hyperactivity and impulsivity, ASD manifests by impairment in the

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development of social skills and communication, and the presence of stereotypical behaviors. The two diagnoses are not mutually exclusive and frequently occur in comorbidity (Sokolova et al. 2017; Zablotsky et al. 2017). Furthermore, these disorders seem to share a common genetic background (Ghirardi et al. 2018; Lichtenstein et al. 2010; Pettersson et al. 2013; Rommelse et al. 2011).

In spite of the high heritability estimates for ADHD (76%) (Brikell et al. 2015; Chang et al. 2013) and ASD (80%) (Ronald and Hoekstra 2011; Tick et al. 2016), and the increasingly larger samples, only a few genome-wide significant hits have been reported, which contribute to only a small part of the overall heritability (Autism Spectrum Disorders Working Group of The Psychiatric Genomics Consortium 2017; Demontis et al. 2018). Also, the replication of these findings is inconsistent, rendering the genetic architecture underlying these neurodevelopmental disorders still largely unknown. Apart from this, classical candidate genes have emerged as potential biological substrates involved in the development of ADHD and ASD, and some of these genes presented pleiotropic effects being associated with both disorders (Akutagava-Martins et al. 2016; Chen et al. 2015; Gilbert and Man 2017; Hawi et al. 2015).

Neurodevelopmental processes are essential to brain functioning, requiring highly regulated cell guidance events (Jackson et al. 2016). These processes depend on a small number of cell guidance receptors and are influenced by genetic variation (Tau and Peterson 2010). For instance, the dysregulation of axon guidance and neurite outgrowth processes were suggested to be involved in ADHD etiology (Poelmans et al. 2011) and more recently in ASD as well (Gilbert and Man 2017). Specific genes involved in these processes have been largely studied in neurodevelopmental disorders. Among those, a promising candidate gene involved in a range of functions related to the development of the central nervous system (CNS) is *ADGRL3*. This gene encodes a protein shown to modulate interactions between adjacent neurons, thus affecting axon guidance, synaptogenesis and synaptic plasticity (Meza-Aguilar and Boucard 2014).

ADHD and ASD present a pattern of biased prevalence towards males. The underlying reasons for this phenomenon are poorly understood, but current evidence points to an involvement of brain morphology differences (Mottron et al. 2015; Park et al. 2018; Ruigrok et al. 2014), brain maturational delays (Koolschijn and Crone 2013), differential effects of sex-hormones and sex-chromosomes (Davies 2014; Davies and Wilkinson 2006; Loke et al. 2015; Mitra et al. 2016), neuroinflammation (McCarthy 2016), and sex-biased DNA methylation (Nugent et al. 2015) and gene expression (Loke et al. 2015; Ruigrok et al. 2014). The differential prevalence of these disorders and the evidence for distinct patterns of brain morphology and function between males and females raise the possibility of sex-specific underlying genetic factors (Gobinath et al. 2017). Recent studies point to a multifactorial liability threshold model, with a protective effect in females, derived from the need to present a greater burden of genetic risk in order to manifest either ADHD or ASD (Gilman et al. 2011; Jacquemont et al. 2014; Martin et al. 2017; Taylor et al. 2016) and also suggest the presence of sex-genotype interactions (Mitra et al. 2016).

ADGRL3 expression is highly specific and regulated during brain development, peaking in the prenatal and postnatal periods (Arcos-Burgos et al. 2010; Haitina et al. 2008; Xing et al. 2009). Its important role in synaptic development and the timely expression pattern suggest that disruptions in gene expression or protein functioning affect individuals earlier in life and produce persistent effects throughout life. Noteworthy, the protein is expressed in brain areas known to present differential volume measures according to sex (Ruigrok et al. 2014) and to be involved in ADHD and ASD, such as the amygdala, cerebellum and cerebral cortex (Arcos-Burgos et al. 2010). In this sense, the implications of *ADGRL3* regulatory role in CNS developmental mechanisms in the emergence of neurodevelopmental psychiatric conditions are especially relevant in the context of male neurodevelopment.

Furthermore, *ADGRL3* has been repeatedly associated with ADHD in several samples, from different clinical and genetic backgrounds (Acosta et al. 2016; Arcos-Burgos et al. 2010; Bruxel et al. 2015; Choudhry et al. 2012; Gomez-Sanchez et al. 2016; Hwang et al. 2015; Ribasés et al. 2011). These findings are also supported by genome-wide linkage findings (Arcos-Burgos et al. 2004) and by animal models that implicate *ADGRL3* in ADHD pathophysiology (Lange et al. 2012; Orsini et al. 2016; Reuter et al. 2016; van der Voet et al. 2015; Wallis et al. 2012). One specific variant (rs651665) has been extensively studied and associated with ADHD in a meta-analysis (Arcos-Burgos et al. 2010). This variant was also shown to influence the severity of ADHD symptoms and the response to treatment with stimulants (Arcos-Burgos et al. 2010; Bruxel et al. 2015; Choudhry et al. 2012). Interestingly, these results derive from studies of ADHD in children, while replication attempts in adults have not retrieved the same findings (Arcos-Burgos et al. 2010; Kappel et al. 2017).

Considering previous evidence implicating variants from *ADGRL3* in ADHD, mostly in its early-onset form, its role in the proper development of the central nervous system, its differential expression considering developmental periods and sexually dimorphic brain regions, and the substantial shared genetic risk between neurodevelopmental disorders, we aimed to assess the influence of *ADGRL3*—rs6551665 in two acknowledged neurodevelopmental disorders: males with early-onset ADHD and boys with ASD.

Methods

Subjects

ASD Sample

The ASD sample consists of 135 male patients recruited at the Hospital de Clínicas de Porto Alegre (HCPA) and/or other medical and educational institutions of the State of Rio Grande do Sul, Brazil (Schuch et al. 2014). Individuals were diagnosed with idiopathic ASD by experienced and trained neuropediatricians in regular appointments (on average 3–4 appointments) according to the DSM-IV criteria (APA 1994). Exclusion criteria were the presence of Fragile X syndrome or other genetic syndromes, chromosomal abnormalities and lesional abnormalities of the CNS. All individuals included were white Brazilians of European descent. Mean age of ASD patients was 9.1 years (SD = 4.5). The complete research protocol has been described elsewhere (Longo et al. 2009; Schuch et al. 2014).

ADHD Sample

The ADHD sample comprises 187 adult males (18 years or older), recruited from the Adult Division of the ADHD Outpatient Clinic at the Hospital de Clínicas de Porto Alegre (HCPA). Individuals were diagnosed with ADHD by trained psychiatrists through the application of clinical and semi-structured interviews following DSM-IV criteria for ADHD (APA 1994). Individuals included in this study fulfilled full DSM-IV criteria, including the age of ADHD onset before 7 years of age, confirmed with collateral information. Exclusion criteria were the age of ADHD onset between 7 and 12 years old, clinically significant history of neurological disease (e.g., delirium, dementia, epilepsy, head trauma, multiple sclerosis), past or present symptoms of psychosis and an estimated IQ < 70. All individuals included were white Brazilians of European descent, and the average age at assessment was 31.9 years (SD = 10.6).

Control Subjects

A sample of 468 adult males with negative screening for ADHD in the 6-question Adult ADHD Self-Rated Scale Screener (ASRS) (Kessler et al. 2005) and no diagnosis of ASD composes our control group. Individuals were blood donors recruited at the same hospital where all cases were ascertained. Control subjects average age was 31.8 years (SD = 10.5).

The ethics committee of HCPA approved the study, and informed consent was obtained from all subjects or legal representatives.

Genotyping

DNA was extracted from peripheral blood by salting out (Lahiri and Nurnberger Jr 1991). One SNP (rs6551665—Chr 4:61873823, A/G—intronic) in the *ADGRL3* gene was selected and genotyped by TaqMan allelic discrimination assays (Applied Biosystems) according to the manufacturer's suggested protocol. This polymorphism was selected according to previous evidence of genetic association with ADHD (meta-analysis in Arcos-Burgos et al. 2010) and minor allele frequency in the European population above 15%. In order to assure genotyping quality control, approximately 10% of the sample was re-genotyped with no inconsistencies found. Allelic frequencies are in Hardy–Weinberg equilibrium in all samples. Analyses considering the main effect of *ADGRL3*—rs6551665 and interactions with SNPs in the *NCAM1-TTC12-ANKK1-DRD2* cluster in ADHD have been reported previously (Kappel et al. 2017).

Statistical Analyses

Logistic regression analyses were performed to assess the effects of *ADGRL3*—rs6551665 regarding both ADHD and ASD susceptibility, and to general susceptibility to neurodevelopmental disorders (i.e., ADHD and ASD combined). Given the sample size and allele frequencies, a dominant model, where heterozygotes were combined with the less frequent homozygotes, was used for these analyses.

In Silico Functionality Prediction

In order to explore the potential effects of the intronic variant rs6551665 in the functionality of *ADGRL3*, we performed an in silico prediction of regulatory mechanisms involved in gene expression. We interrogated the publicly available regulatory databases—HaploReg v4.1 (Ward and Kellis 2012) and RegulomeDB (Boyle et al. 2012). HaploReg is a web-based database aimed at exploring the impact of noncoding variants on clinical phenotypes through its effects on gene expression regulatory mechanisms. It contains information regarding chromatin state, histone modifications and protein binding annotation. Similarly, RegulomeDB annotates SNPs with known and predicted regulatory elements in intergenic regions; some of its integrated data overlaps with HaploReg but it also generates a score (range 1–7) suggesting the likelihood of regulatory evidence for each locus.

Results

We observed an association between *ADGRL3*—rs6551665 and ADHD susceptibility in males with early onset of ADHD symptoms. The presence of the minor G allele (i.e., AA vs. AG + GG) was associated with an increased risk of ADHD in this sample ($p = 0.025$; OR 1.502) (Table 1).

Additionally, in the susceptibility study in boys with ASD, we also observed an association of *ADGRL3*—rs6551665 and the disorder. A similar pattern to the one observed in ADHD arises, where the presence of at least one minor G allele confers increased risk to ASD development ($p = 0.044$; OR 1.511) (Table 1).

Finally, as expected based on the analyses with each disorder, in the combined analysis of both neurodevelopmental disorders we observed a stronger association with the presence of the minor G allele in *ADGRL3*—rs6551665 ($p = 0.007$; OR 1.500) (Table 1).

We explored the effects of rs6551665 on *ADGRL3* through an in silico prediction analyses. HaploReg suggests that rs6551665 is involved in gene expression regulatory mechanisms especially in brain tissues, consistent with the fact that *ADGRL3* is mainly expressed in the brain. Chromatin state data suggest that rs6551665 is related to enhancer activity in almost all brain tissues evaluated (hippocampus, substantia nigra, inferior temporal lobe, angular gyrus, and germinal matrix). Furthermore, this analysis also suggests that the SNP appears to be associated with an active transcription initiation site in the anterior caudate brain region (Supplementary file 1). Different histone marks are detected at rs6551665 locus (H3K4me1, H3K4me3, H3K27ac and H3K9ac); the presence of H3K27Ac, often found near regulatory elements, suggests that the loci could have a role as regulatory enhancer region. This set of results is also observed in RegulomeDB; the database score of 5 suggests that rs6551665 is placed in an enhancer region but has an overall minimal transcription factor binding evidence.

Discussion

The results presented here suggest that *ADGRL3* could be part of a common genetic etiology for ADHD and ASD, being implicated in the underlying neurobiology of two common neurodevelopmental disorders. These results derive from an integrative approach that acknowledges the substantial shared genetic risk between psychiatric disorders and highlight the importance of study designs that accommodate and investigate specific overlapping genetic factors that can give rise to a range of diagnoses.

Table 1 Logistic regression analyses regarding the influence of *ADGRL3*—rs6551665 on ADHD and ASD

	Genotype	Controls *n* (%)	Neurodevelopmental disorders[b] *n* (%)	Odds Ratio (CI 95%)[a]	*p*-value	ADHD *n* (%)	Odds Ratio (CI 95%)[a]	*p*-value	ASD n (%)	Odds Ratio (CI 95%)[a]	*p*-value
ADGRL3—rs6551665	AA	197 (42.1)	105 (32.6)	1.500 (1.120–2.020)	0.007	61 (32.6)	1.502 (1.051–2.145)	0.025	44 (32.6)	1.511 (1.009–2.263)	0.044
	G-carriers	271 (57.9)	217 (67.4)			126 (67.4)			91 (67.4)		

ADHD Attention-Deficit/Hyperactivity Disorder, *ASD* Autism Spectrum Disorder

[a]Confidence interval 95%

[b]Neurodevelopmental disorders (combined group with ADHD or ASD)

The overall evidence and present findings may be useful to interpret *ADGRL3* influence in neurodevelopmental conditions. *ADGRL3's* linkage (Arcos-Burgos et al. 2004) and association (Acosta et al. 2016; Arcos-Burgos et al. 2010; Bruxel et al. 2015; Choudhry et al. 2012; Gomez-Sanchez et al. 2016; Huang et al. 2018; Hwang et al. 2015; Ribasés et al. 2011) in childhood ADHD has been extensively demonstrated; we provide further evidence that this gene is involved in the neurobiological substrate of the disorder in a sample of adult males with early-onset ADHD. This result is especially interesting since these subjects comprise a sample with an early-onset of ADHD symptoms and a stable clinical phenotype throughout adulthood, characterizing a classical neurodevelopmental disorder (Thapar et al. 2015). Although not formally investigated, the suggestive involvement of *ADGRL3* in ASD neurobiology observed in this study is not entirely new. A copy number variation investigation in a child with ASD detected a large duplication in the region encompassing the gene (Gau et al. 2012). Interestingly, this same individual presented several symptoms of inattention and hyperactivity. Moreover, a linkage study showed a significant signal for the *ADGRL3* genomic region in dyslexia, another neurodevelopmental disorder (Field et al. 2013).

The influence of *ADGRL3* in neurodevelopmental disorders could be related to its role in synapse development. *ADGRL3* encodes a transmembrane adhesion G protein-coupled receptor, having an especially important function during synaptic development (O'Sullivan et al. 2014). Through its olfactomedin-like domain, ADGRL3 interacts with fibronectin leucine-rich repeat transmembrane 3 protein (FLRT3), forming a trans-synaptic complex (O'Sullivan et al. 2012, 2014). This binding is required to support glutamatergic synapse development, and therefore, it is implicated in the development of the nervous system. The formation of these complexes suggests that ADGRL3 is responsible for establishing accurate glutamatergic signaling during development. Another interesting point is that glutamatergic neurotransmitter system variants and genes have been implicated in both ADHD and ASD (Akutagava-Martins et al. 2014; Chiocchetti et al. 2014, 2018; Elia et al. 2011; Hadley et al. 2014; Mariani et al. 2015; Naaijen et al. 2017; Noroozi et al. 2016; Uzunova et al. 2014). In this sense, the effects of ADGRL3 in the formation of these specific synapses, through its interactions with FLRT3, could be a potential mechanism related to the neurodevelopmental origins of ADHD and ASD.

These results should be taken in the context of some limitations. Firstly, only the new DSM-5 criteria allow the comorbidity of ADHD and ASD in index cases of both disorders. The samples of this study were collected under DSM-IV criteria, a classificatory system that did not allow the presence of ADHD-ASD comorbidity. Future studies should address the association of both disorders and investigate whether the effect size of the association reported here could be even stronger in individuals diagnosed with both disorders. Secondly, the control group was not age-paired with the individuals with ASD, but they did not present a diagnosis of neither ADHD nor ASD until adulthood. Also, our study design, comprising only male samples of ADHD and ASD, hinders the extrapolation of the present results to female samples. However, since a previous study did not detect a main effect in ADHD (Kappel et al. 2017), it is unlikely that this variant might have a significant role in this group. Similarly designed studies with larger sample sizes and also evaluating these genetic effects in females with autism are necessary. Finally, *ADGRL3*—rs6551665 is an intronic variant and its exact molecular effect and consequent role in biological mechanisms is still unknown. A screening of potential mutations in the *ADGRL3* gene in patients with ADHD revealed that its effect is most likely caused by a partial reduction of gene activity, and not by a complete loss of function (Domené et al. 2011). Therefore, the *ADGRL3* variant studied herein (rs6551665) could be exerting its effects through regulatory mechanisms related to gene expression as suggested by the in silico predictions. According to previous work (Domené et al. 2011), we could hypothesize that subtle changes in protein expression and availability influence underlying neurobiological mechanisms through ADGRL3 direct role or through the trans-synaptic complex formation with FLRT3 (O'Sullivan et al. 2012). Alternatively, considering that several studies have identified other *ADGRL3* variants also associated with neurodevelopmental disorders (Acosta et al. 2016; Bruxel et al. 2015; Choudhry et al. 2012; Huang et al. 2018; Martinez et al. 2016; Ribasés et al. 2011), it is also possible that we are capturing the by-proxy effect of another functional variant in linkage disequilibrium.

In conclusion, these results add to the increasing amount of knowledge depicting the role of neurodevelopmental genes in the substrate of ADHD and ASD. The overall state-of-the-art suggests that *ADGRL3* might be a gene deeply involved in the development of the brain, and its genetic modifications might be part of a common vulnerability factor related to neurodevelopmental disorders, which in turn can manifest, depending on the remaining genetic background as different disorders like ADHD and ASD. Nevertheless, the evidence presented here warrants the need for future studies that acknowledge and investigate *ADGRL3* pleiotropic effects and possible influence in other neuropsychiatric and neurodevelopmental disorders.

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Compliance with Ethical Standards

Conflict of interest The author(s) declare the following potential conflict of interest with respect to the research, authorship and/or publication of this article: Dr. Grevet was on the speaker's bureau for Novartis and Shire for the last 3 years. Dr. Rohde has received Honoraria, has been on the speakers' bureau/advisory board and/or has acted as a consultant for Eli-Lilly, Janssen-Cilag, Medice, Novartis and Shire in the last three years. He receives authorship royalties from Oxford Press and ArtMed. The ADHD and Juvenile Bipolar Disorder Outpatient Programs chaired by him received unrestricted educational and research support from the following pharmaceutical companies in the last three years: Eli-Lilly, Janssen-Cilag, Novartis, and Shire. All other authors report no biomedical financial interests or potential conflicts of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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