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# *NOS1* and *SNAP25* polymorphisms are associated with Attention-Deficit/Hyperactivity Disorder symptoms in adults but not in children

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ABSTRACT

Several investigations documented that Attention-Deficit/Hyperactivity Disorder (ADHD) is better conceptualized as a dimensional disorder. At the same time, the disorder seems to have different neurobiological underpinnings and phenotypic presentation in children compared to adults. Neurodevelopmental genes could explain, at least partly these differences. The aim of the present study was to examine possible associations between polymorphisms in *SNAP25*, *MAP1B* and *NOS1* genes and ADHD symptoms in Brazilian samples of children/adolescents and adults with ADHD. The youth sample consisted of 301 patients whereas the adult sample comprises 485 individuals with ADHD. Diagnoses of ADHD and comorbidities were based on the Diagnostic and Statistical Manual of Mental Disorders–4th edition criteria. The Swanson, Nolan and Pelham Scale-Version IV (SNAP-IV) was applied by psychiatrists blinded to genotype. The total SNAP-IV scores were compared between genotypes. Impulsivity SNAP-IV scores were also compared according to *NOS1* genotypes. Adult patients homozygous for the *C* allele at *SNAP25 rs8636* showed significantly higher total SNAP-IV scores ($F = 11.215$; adjusted $P$-value $= 0.004$). Impulsivity SNAP-IV scores were also significantly different according to *NOS1 rs478597* polymorphisms in adults with ADHD ($F = 6.282$; adjusted $P$-value $= 0.026$). These associations were not observed in children and adolescents with ADHD. These results suggest that *SNAP25* and *NOS1* genotypes influence ADHD symptoms only in adults with ADHD. Our study corroborates previous evidences for differences in the genetic contribution to adult ADHD compared with childhood ADHD.



## 1. Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized by inappropriate levels of hyperactivity, impulsivity and inattention. ADHD is highly influenced by genetic factors; its heritability was estimated around 76% in children (Faraone et al., 2005). More recently, high heritability of attention problems as indexed by parent and self-ratings from childhood to early adulthood ($h^2 = 0.77–0.82$) was also estimated in the Swedish Twin Study of Child and Adolescent Development cohort (Chang et al., 2013). However, the genetic architecture of this disorder remains unknown partially due to its complex etiology and clinical heterogeneity. Molecular genetic studies have suggested that multiple common and rare genetic variants could be involved in ADHD (Martin et al., 2015).

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Although the clinical utility of categorically defined ADHD is well established (Biederman, 2005), there is also strong evidence supporting the notion of ADHD as an extreme of a continuous trait (Bralten et al., 2013; Larsson et al., 2012; Martin et al., 2014; Salum et al., 2014; Shaw et al., 2013; Stergiakouli et al., 2015). Larsson et al. (2012) based on a large twin study suggested that ADHD is best viewed as the quantitative extreme of genetic and environmental factors operating dimensionally throughout the distribution of ADHD symptoms, indicating that the same etiologic factors are involved in the full range of symptoms of inattention, hyperactivity and impulsivity. Even though latent classes derived from quantitative symptom counts have been shown to be heritable and may offer greater gene discovery potential than DSM-defined subtypes, there has been little application of these symptom-based approaches within ADHD genetic studies (Rasmussen et al., 2004). In a recent revision, Hawi et al. (2015) pointed that the biological understanding of ADHD is limited by several factors: the small size of individual gene effects; the heterogeneity of genetic effects; reduced gene penetrance and the presence of phenocopies within the sample. For these reasons, studies of susceptibility genes for psychiatric disorders have emphasized the utility of quantitative indices of disease risk instead of categorical diagnosis.

Although the proportion of persistence of ADHD diagnosis from child to adulthood is still in debate, the majority of children with ADHD will have substantial symptoms of the disorder into adulthood (Barkley et al., 2002; Haavik et al., 2010; Karam et al., 2015; Matte et al., 2012). Strikingly, main features of adult ADHD differ from typical ADHD in children (Franke et al., 2012; Volkow and Swanson, 2013). The prevalence of ADHD in childhood is estimated as 5.3% (Polanczyk et al., 2007), whereas this rate in adults is among 2.5–4.4% (Kessler et al., 2006; Simon et al., 2009). The gender ratio in children with ADHD differs from adults. Boys have a higher prevalence than girls in childhood, but this ratio is quite similar in adulthood (Biederman and Faraone, 2004; Grevet et al., 2006). During childhood, disruptive behaviors are the most prevalent comorbities in ADHD, whereas the comorbid profile in adulthood is more complex (McGough et al., 2005). Moreover, the decrease in hyperactive/impulsive symptoms over time is pronounced during childhood and adolescence suggesting that ADHD could not be viewed as a stable disorder (Larsson et al., 2011; Matte et al., 2012). Different abnormalities in brain structures were observed between children and adult ADHD presentations (Friedman and Rapoport, 2015). Moreover, some genetic findings are restricted either to children samples or to adult samples. This dissimilarity may represent differences in genetic susceptibility to adult ADHD compared with those linked to the disorder in childhood (Franke et al., 2012; Salatino-Oliveira et al., 2015).

Pathway analyses have suggested genes involved into neurodevelopmental processes as candidates for ADHD genetic susceptibility investigations (Elia et al., 2010; Poelmans et al., 2011; Yang et al., 2013). Among those genes, the *MAP1B* that encodes the microtubule-associated protein 1B was included. This protein could act in several different categories of cellular functions, such as expression of glutamatergic systems, neuronal migration, myelination, axon guidance and *corpus callosum* formation (Gonzalez-Billault et al., 2000; Meixner et al., 2000; Moritz et al., 2009; Villarroel-Campos and Gonzalez-Billault, 2014). Other potential candidate is the nitric oxide synthase 1 (*NOS1*) that has been pointed as a relevant gene mainly for ADHD impulsivity symptoms (Hawi et al., 2015; Hoogman et al., 2011; Reif et al., 2009). NOS1 enzyme bounds to N-methyl-D-aspartate receptors (NMDAr) and produces nitric oxide (NO; Lesch et al., 2013). Moreover, NOS1 seems to influence cadherin systems and could play a role in neurite outgrowth (Chen et al., 2006; Poelmans et al., 2011). *SNAP25* encodes the synaptosomal-associated protein of 25 kDa, a presynaptic plasma membrane protein which belongs to the SNARE complex (Jahn and Scheller, 2006). *SNAP25* seems to play an important role in axonal growth, synaptic plasticity and neurotransmitter release, acting in essential steps for wiring the nervous systems (Antonucci et al., 2013). Several approaches suggested that *SNAP25* is a promising susceptibility locus for ADHD (Chang et al., 2012; Forero et al., 2009; Gizer et al., 2009; Guan et al., 2009; Hawi et al., 2015; Lasky-Su et al., 2008).

Overall, the ADHD dimensional approach is suggested to be better than categorical ones for genetic association analyses. Moreover, differences in neurodevelopmental genes probably could help to understand differences between children and adults with ADHD. Therefore, the aim of the present study was to examine the possible association between polymorphisms in *SNAP25*, *MAP1B* and *NOS1* genes and ADHD symptoms in children and adults with ADHD.

## 2. Methods

### 2.1. Subjects

The youth sample consisted of 301 children and adolescents recruited at the ADHD Outpatient Program (ProDAH) from Hospital de Clínicas de Porto Alegre. ADHD was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (American Psychiatric Association, 1994). It followed a previously reported three-staged protocol (Salatino-Oliveira et al., 2012), including the application of semi-structured diagnostic interviews (KSADS-PL) by trained research assistants and clinical assessments by experienced child psychiatrists. The Swanson, Nolan and Pelham Scale-Version IV (SNAP-IV) was applied by child psychiatrists blinded to genotype to assess phenotypic presentations. This scale is based on DSM-IV ADHD symptoms and assesses ADHD core symptoms of hyperactivity, impulsivity and inattention, along with oppositional symptoms since it is often present in patients with ADHD (The MTA, 1999).

The adult sample comprises 485 patients that were recruited at the adult division of the ProDAH. The diagnoses for ADHD and comorbidities also followed the DSM-IV criteria. ADHD and oppositional defiant disorder diagnoses followed the same three-staged procedure described above for children and adolescents. Questions from Schedule for Affective Disorders and Schizophrenia for School-Age Children, Epidemiological Version (K-SADS-E) designed for children were adapted for adults (Grevet et al., 2005). Axis I psychiatric comorbidities were evaluated using the Structured Clinical Interview for DSM-IV, research version (SCID-I-RV) (First, 1998). The diagnoses of conduct disorder and anti-social personality disorder were obtained using the appropriate sections of the Mini International Neuropsychiatric Interview (MINI) (Amorim, 2000). The SNAP-IV questionnaire was also applied to these subjects.

This study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre. Adults were invited to participate and provided a written informed consent. For the children/adolescents, the parents provided written informed consent and probands provided verbal assent to participate.

### 2.2. SNP selection and genotyping

The *SNAP25 rs8636* single nucleotide polymorphism (SNP) is mapped to the 3' untranslated region (UTR). It was chosen due to its previous positive association with ADHD (Guan et al., 2009; Sarkar et al., 2012). This SNP has possible interactions with miRNAs as indicated by the Functional SNP Prediction tool (Xu and Taylor, 2009; available in http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.