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Cadherin-13 Gene is Associated with Hyperactive/Impulsive Symptoms in Attention/Deficit Hyperactivity Disorder

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Several efforts have been made to find new genetic risk variants which explain the high heritability of ADHD. At the genome level, genes involved in neurodevelopmental pathways were pointed as candidates. *CDH13* and *CTNNA2* genes are within GWAS top hits in ADHD and there are emerging notions about their contribution to ADHD pathophysiology. The main goal of this study is to test the association between SNPs in *CDH13* and *CTNNA2* genes and ADHD across the life cycle in subjects with ADHD. This study included 1,136 unrelated ADHD cases and 946 individuals without ADHD. No significant association between *CDH13* and *CTNNA2* was observed between cases and controls across different samples ($P \geq 0.096$ for all comparisons). No allele was significantly more transmitted than expected from parents to ADHD probands. The *CDH13 rs11150556 CC*

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genotype was associated with more hyperactive/impulsive symptoms in youths with ADHD (children/adolescents clinical sample: F = 7.666, *P* = 0.006, FDR *P*-value = 0.032; Pelotas Birth Cohort sample: F = 6.711, *P* = 0.011, FDR *P*-value = 0.032). Although there are many open questions regarding the role of neurodevelopmental genes in ADHD symptoms, the present study suggests that *CDH13* is associated with hyperactive/impulsive symptoms in youths with ADHD.

Key words: **neurodevelopment; CDH13; CTNNA2; cadherins; ADHD**

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder, as defined by the Diagnostic and Statistical Manual of Mental Disorders - fifth edition (DSM-5, American Psychiatry Association, 2013). The ADHD is characterized by a pattern of age-inappropriate behavior symptoms divided into two dimensions: inattention and hyperactivity/impulsivity. Although the diagnostic criteria distinguish three different presentations of ADHD according to predominant symptoms, the disorder can be better understood dimensionally [Hudziak, 2007; Kraemer, 2007; Salum et al., 2014]. This disorder is one of the most prevalent mental health disorders in childhood and adolescence, affecting 5.29% of children worldwide [Polanczyk et al., 2007]. Even though the proportion of persistence and prevalence rates of ADHD in adults are still controversial [Matte et al., 2014], there are studies suggesting that up to 66% of ADHD children will have clinically significant symptoms of the disorder in adulthood [Barkley et al., 2002; Haavik et al., 2010].

ADHD is a very heterogeneous disorder and its etiology remains unclear. However, several studies support a strong genetic contribution. A meta-analysis of twin studies estimated an average ADHD heritability of 76% [Faraone et al., 2005]. Although ADHD is highly heritable, few variants with small effect were identified that explain a small portion of the estimated heritability. Several genome-wide association (GWAS) studies have been conducted. Despite without statistically significant results at the genome level, genes related to neurotransmission and cell-cell communication systems were suggested, including genes related to processes such as cell division, adhesion and polarity, neuronal migration and plasticity, extracellular matrix regulation, and cytoskeletal remodeling [Poelmans et al., 2011]. These findings from genome-wide approaches indicate a whole range of new and promising possibilities for ADHD molecular genetic studies [Akutagava-Martins et al., 2013]. A pathway-based analysis using case-only design showed that neurite outgrowth genes pointed by Poelmans et al. (2011) were associated with hyperactive/impulsive scores in ADHD children from the International Multicenter ADHD Genetics (IMAGE) study [Bralten et al., 2013].

The most promising gene derived from these studies is *CDH13* which was among top hits of three independent investigations [Lasky-Su et al., 2008; Lesch et al., 2008; Neale et al., 2010]. This gene is located at chromosome *16q23.3* and encodes cadherin-13 which belongs to a large family of transmembrane adhesion calcium-dependent molecules [Philippova et al., 2009]. However, the cadherin-13 protein is an atypical cadherin. It lacks transmembrane and cytosolic domains and it is attached to the cellular membrane by a glycosylphosphatidylinositol (GPI) anchor [Philippova et al., 2009]. This characteristic of cadherin-13 allows this protein to have a critical role as a signaling molecule differently from classical cadherins [Rivero et al., 2013]. The CDH13 transcripts were found in prefrontal cortex (PFC), medulla, thalamus, and midbrain of the human adult brain whereas the protein was detected in cerebral cortex, *medulla oblongata* and *nucleus olivaris* cellular membranes and neurites [Takeuchi et al., 2000]. The CDH13 functions could be potentially related to neurite outgrowth, dendrite arborizations, synapse development, and maintenance of synaptic contacts [Rivero et al., 2013].

Two candidate gene studies in independent samples were performed with *CDH13* and ADHD. The first tested the association between this gene and three executive function tasks. The *rs11150556* polymorphism was associated with verbal working-memory (VWM) performance in children with ADHD. Carriers of the *CC* genotype showed a significant worse performance when compared to *CT* and *TT* carriers [Arias-Vásquez et al., 2011]. The second study aimed to identify, characterize and estimate coding *CDH13* variants in adults with ADHD [Mavroconstanti et al., 2013]. Seven coding variants were found; only one was novel and none of them showed significant associations with ADHD. Moreover, no significant differences were observed between expression levels of these coding variants in either HEK293 or CHO cells [Mavroconstanti et al., 2013].

Another gene involved in neurodevelopmental pathways that figures at the GWAS top hits is the *CTNNA2* [Poelmans et al., 2011]. This gene encodes the αN-catenin protein and is located at chromosome *2p12-p11.1* [Kobielak and Fuchs, 2004]. The α-catenins binds typical cadherins with the actin cytoskeleton and it maintains the stability of dendritic spines and synaptic contact [Abe et al., 2004]. The αN-catenin is highly expressed in the central nervous system, particularly in hypothalamus, amygdala, cingulate cortex, temporal lobe, and PFC [Terracciano et al., 2011]. Adhesion junctions in border active zones in developing and mature synapses have active αN-catenin throughout the developing and postnatal brain [Terracciano et al., 2011]. αN-catenins seems to stabilize synapse formation mediated by typical cadherins during the development of nervous system. Further studies have supported the involvement of *CTNNA2* in ADHD etiology. The *CTNNA2* gene is located within CNVs in individuals with ADHD [Elia et al., 2010]. Moreover, a GWAS identified an association between scores in the excitement-seeking scale of the revised Neuroticism-Extraversion-Openness personality inventory and *CTNNA2* gene. This trait is a main component of impulsivity and it is a core feature of extraversion [Terracciano et al., 2011]. This result suggests a role of the *CTNNA2* gene in the biological basis of the excitement-seeking and impulsive behaviors that are common in patients with ADHD [Terracciano et al., 2011].

Therefore, there are emerging data about the *CDH13* and *CTNNA2* contribution to the pathophysiology of ADHD. Candidate gene studies in independent samples are required in

order to validate GWAS top-findings and to determine the role of these genes in ADHD etiology. The main goal of this study was to test the association between single nucleotide polymorphisms (SNPs) in *CDH13* and *CTNNA2* genes and ADHD across the life cycle in subjects with ADHD.

MATERIAL AND METHODS

Subjects

This study included a total of 1,136 unrelated ADHD cases and 946 individuals without ADHD who came from three sources where the phenotype was extensively assessed. The first sample consisted of 526 ADHD children and adolescents and their biological parents. These patients were recruited at the ADHD Outpatient Program (ProDAH) from Hospital de Clínicas de Porto Alegre. The sample was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria [American Psychiatric Association, 1994], following a previously reported three-stage protocol [Roman et al., 2001], including the application of a semi-structured diagnostic interviews (KSADS-PL) and clinical assessments by experienced child psychiatrists. The Swanson, Nolan, and Pelham Scale-Version IV (SNAP-IV) was applied to 277 of those patients by child psychiatrists blinded to genotype. A control group of 129 children and adolescents without evidence of any current or past mental illness as defined by the DSM-IV was selected. The diagnostic approach for the control sample was the same as for the ADHD samples [Schmitz et al., 2006].

The second ADHD sample comprises 110 individuals from the 1993 Pelotas Birth Cohort. These subjects were evaluated at 18–19 years of age in a one-stage procedure. A structured interview based on the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) was performed [American Psychiatric Association, 2013]. The ADHD in adults in DSM-5 is defined as the presence of at least five among nine symptoms of inattention and/or five among nine symptoms of hyperactivity/impulsivity. ADHD symptoms must cause clinical interference, several of them must be present in more than one setting, and their age of onset should be before 12 years of age. The DSM-5 ADHD symptoms were rated as present or absent. Initially, a screening questionnaire using the same structure of the six-question World Health Organization Adult ADHD Self-Report Scale Screener (ASRS) was applied for all subjects. This screening consists of six questions about ADHD symptoms (two hyperactivity items: "on the go", and "fidgets"; and four inattention items: "forgetful", "does not follow through", "reluctant to engage in 'mental' tasks", and "difficulty organizing tasks"), which were adapted to the DSM-5 wording. Considering a cut-off of 4/6 screening symptoms, ASRS had 68.7% sensitivity, 99.8% specificity, and 97.9% total classification accuracy in a previous population study [Kessler et al., 2005]. In order to enhance sensitivity, any subject with two or more positive questions among the six was considered screening positive in our study. In this case, they answered questions about the 12 remaining ADHD symptoms, as well as about additional criteria (clinical impairment, symptom pervasiveness, and age of onset before 12 years old). Pervasiveness was assessed by questioning if the subject presented symptoms in at least two of the three main settings: home, social, and work/school environments. To measure the clinical impairment specifically related to ADHD, a scale ranging from 0 (no impairment) to 3 (severe impairment) was applied at the end of the ADHD assessment interview. Clinical impairment was defined as scores of 2 (moderate) or 3 (severe). A total of 508 individuals were selected from the 1993 Pelotas Birth Cohort as controls. This control sample includes all subjects with positive screening (i.e., at least two positive questions in the six question screening) who did not meet full ADHD diagnostic criteria in the subsequent evaluation ($n = 179$) and individuals with none of the six ADHD screening symptoms ($n = 331$; for more details see [Matte et al., 2014]).

Methodology of data collection and demographic data from this Cohort are described fully in Victora et al. (2006) and Matte et al. (2014). The Mini International Neuropsychiatric Interview (MINI) was performed to evaluate several other psychiatric diagnoses. The MINI is a short semi-structured diagnostic interview for DSM-IV and ICD-10 psychiatric disorders, which provides prevalence estimates of common mental disorders. Only some MINI sections were used due to logistic issues (i.e, the psychiatric interview was part of a larger follow-up assessment). The most prevalent mood and anxiety disorders were assessed: bipolar, major depression, agoraphobia, generalized anxiety, and social phobia. The MINI has a previously validated Portuguese version [Amorim, 2003]. The MINI had kappas of 0.65–0.85, sensitivity of 0.75–0.92, and specificity of 0.90–0.99 when using Structured Clinical Interview for Diagnosis (SCID) applied by a psychiatrist as a parameter in primary health care in Brazil [de Azevedo Marques and Zuardi, 2008].

The third ADHD sample comprises 500 adults with ADHD that were also recruited at ProDAH. The diagnostic procedures for ADHD and comorbidities followed the DSM-IV criteria. The ADHD and oppositional defiant disorder diagnoses followed the same three-staged procedure described above for children and adolescents. Questions from 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 et al., 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, 2003]. The adult control group of 309 individuals was evaluated in a blood donation center in the same hospital. The inclusion criteria were: (i) be Brazilian of European descent; and (ii) 18 years of age or older. The exclusion criteria were the same as those used for the ADHD patients (mentioned above), as well as the fulfillment of a DSM-IV ADHD diagnosis. Furthermore, the SNAP-IV questionnaire was also applied.

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

Genotyping

Blood samples were collected from patients enrolled at ProDAH and their parents whenever possible. DNA was extracted from

lymphocytes by standard procedures. DNA samples from the 1993 Pelotas Birth Cohort were obtained from saliva, using Oragene® OG-250 DNA Self-Collection kit, following the manufacturer's recommended protocol (DNA Genotek Inc., Kanata, Ontario, CA). DNA from all samples was quantified by spectrophotometry using NanoDrop 1000 (Thermo Fisher Scientific Inc., Waltham, MA) and diluted to a standard concentration of 10 ng/μL. The polymorphisms *rs11646411*, *rs6565113,* and *rs11150556* in *CDH13* and *rs13395022* in *CTNNA2* genes were genotyped using TaqMan SNP Genotyping Assays-on-Demand (Applied Biosystems, Foster City, CA) according to the manufacturer's recommended protocol. Three SNPS (*CDH13 rs11646411, rs6565113*) and *CTNNA2 rs13395022*) were chosen because they are GWAS top hits [Poelmans et al., 2011]. *CDH13 rs11150556* was included due to its association with verbal working-memory in children with ADHD [Arias-Vásquez et al., 2011].

Statistical Analyses

Allele frequencies were estimated by counting. Deviations from Hardy–Weinberg equilibrium were assessed by the χ^2 test. The PLINK program v.1.07 was used to estimate the *D'* as a measure of pairwise linkage disequilibrium (LD) between SNPs at *CDH13* gene [Purcell et al., 2007]. Comparisons among variables were performed using the χ^2, Fisher's exact or Mann–Whitney U-test (for quantitative variables without normal distributions). Potential confounders to be entered in the models were defined based on conceptual analyses of the literature and by means of a statistical definition (association with the study factor and with the outcome at $P < 0.10$). The association of the SNPs with ADHD susceptibility was tested in a case–control design by multivariate logistic regression analysis in each group. Hyperactivity/impulsivity SNAP-IV scores were compared among genotypes by ANOVA. The effect size for significant *P*-values was obtained through partial eta squared. All these tests were performed with SPSS Version 18 software (SPSS, Inc., Chicago, IL). The family-based association tests were carried out with the UNPHASED v.3.1.7. software [Dudbridge, 2008]. The significance level accepted for all tests was 0.05 and the adjustment for multiple testing by false discovery rate (FDR) procedure was performed [Benjamini and Hochberg, 1995].

RESULTS

Children with ADHD were younger and predominantly males when compared to controls whereas the adults with ADHD were older than their controls. Most individuals are of European ancestry, although some individuals of African ancestry were also included. The most common comorbid disorders observed in these ADHD patients were anxiety, disruptive behavior, and mood disorders (Table I).

Allele frequencies were estimated for each SNP within each group. Genotype distributions were in Hardy-Weinberg equilibrium in all samples (Table SI). The *D'* among SNPs at *CDH13* gene was D' = 0.009 between *rs11646411* and *rs6565113*, D' = 0.062 between *rs11646411* and *rs11150556*, and D' = 0.010 between *rs6565113* and *rs11150556*. These D' values indicated absence of LD among these polymorphisms.

TABLE I. Demographic and Clinical Characteristics of Each Group[a]

	Children/Adolescents from ProDAH			1993 Pelotas birth cohort			Adults from ProDAH		
Characteristics	**Case**	**Control**	***P*-value[b]**	**Case**	**Control**	***P*-value[b]**	**Case**	**Control**	***P*-value[b]**
Age (years)	10.5 ± 3.08	11.6 ± 3.14	0.001	18	18	—	33.6 ± 11.1	30.2 ± 9.1	<0.001
Gender (male)	403 (76.6%)	80(62.0%)	0.001	42 (38.2%)	228(44.5%)	0.205	265 (53.0%)	143(46.3%)	0.071
Ethnicity (European–Brazilian)	409 (77.7%)	98(76.0%)	0.269	69 (62.8%)	353(69.5%)	0.168	500 (100%)	309(100%)	—
Anxiety disorders[c]	120 (22.8%)	25(19.4%)	0.410	55 (50.0%)	55(10.8%)	<0.001	57 (18.4%)	159(31.8%)	<0.001
Disruptive behavior disorders	260 (49.4%)	14(10.9%)	<0.001	—	—	—	266 (53.2%)	6(2.0%)	<0.001
Mood disorders	80 (15.2%)	2(1.6%)	<0.001	23 (20.9%)	13(2.6%)	<0.001	203 (40.6%)	80(25.9%)	<0.001

[a]Data are given as number (percentage) or mean (±standard deviation).
[b]Calculated by the Mann–Whitney U-test (quantitative variables without normal distributions), the χ^2-test (categorical variables).
[c]Agoraphobia, generalized anxiety disorder, social phobia.

The distribution of ADHD among SNP genotypes in *CDH13* and *CTNNA2* genes was analyzed by logistic regression in each case-control sample. No significant association was observed in case-control analyses across the three different samples (Table II). The association of the *rs11150556 CC* genotype with ADHD in the 1993 Pelotas birth cohort was not maintained after FDR correction (F = 1.601, *P*-value = 0.032, FDR *P*-value = 0.096; Table II). Family-based association tests for each investigated variant in the children/adolescents ADHD sample were performed. No allele was significantly more transmitted than expected from parents to ADHD probands ($P \geq 0.211$ for all SNPs; data not shown, but available upon request).

Significant differences in hyperactivity/impulsivity scores in the SNAP-IV between *CDH13 rs11150556* genotype groups in children/adolescents with ADHD (F = 3.901, *P* = 0.011, FDR *P*-value = 0.026; Table III) were observed. Similar differences were found in DSM-5 hyperactivity/impulsivity symptom count in ADHD individuals from the 1993 Pelotas birth cohort (F = 4.116, *P* = 0.022, FDR *P*-value = 0.033; Table III). When *CDH13 CC* carriers were compared to *CT* + *TT* carriers, the difference was more evident. The *CDH13 CC* genotype was associated with higher hyperactive/impulsive symptoms in children/adolescents and young people with ADHD (children/adolescents: F = 7.666, *P* = 0.003, FDR *P*-value = 0.018; Pelotas Birth Cohort: F = 6.711, *P* = 0.013, FDR *P*-value = 0.026; Table III). The corresponding effect sizes were 3% and 7% for children/adolescents and Pelotas birth cohort samples, respectively. This association was not observed in adult patients.

DISCUSSION

The selected polymorphisms at the *CDH13* and *CTNNA2* genes were not associated with ADHD through case-control analyses in all age-different samples. Moreover, family-based tests indicated that there was no over-transmission of any allele of these variants from parents to children/adolescents with ADHD. However, significant-differences in hyperactive/impulsive symptoms in children/adolescents and young people with ADHD according to the *CDH13 rs11150556* genotypes were observed. These results suggest that *CDH13 rs11150556 CC* genotype could lead to increased hyperactive/impulsive symptoms in the early period of the life cycle of ADHD individuals.

Our findings evoke three interesting issues: (i) what would be the reasons for positive findings in hyperactive scores only during childhood and adolescence/young adulthood? (ii) Why only positive findings in dimensional scores and not in categorical diagnosis? (iii) Why positive findings in hyperactive/impulsive dimension and not in inattentive scores?

Although ADHD in children and adolescents often persists into adulthood, the symptomatic presentation of the disorder at later age phases differs from that in children [Volkow and Swanson, 2013]. A fraction of this difference is due to a greater decrease in hyperactive/impulsive symptoms than in inattentive symptoms [Matte et al., 2012; Willcutt et al., 2012; Volkow and Swanson, 2013]. The developmental instability of ADHD symptoms across the life cycle could be reflected by the diagnostic changes in DSM-5, which eliminated the previous enumeration of three "subtypes" of ADHD [Volkow and Swanson, 2013]. Genetic and environment contributions to the developmental course and outcomes of ADHD in adulthood are relatively understudied and more investigations are needed [Franke et al., 2012]. Interestingly, even though a high heritability of ADHD in children is clearly found, some studies estimate a low heritability for ADHD symptoms in adults [Franke et al., 2012] suggesting different etiologic pathways for different ages ranges. Furthermore, the cortical thickness trajectories seem to differ according to ADHD remission or persistence into adulthood

TABLE II. Logistic Regression of the SNPs in CDH13 and CTNNA2 Genes With presence of ADHD Across the Three Groups

			Children/adolescents from ProDAH[a]		1993 Pelotas birth cohort		Adults from ProDAH[a]	
SNP	**Gene**	**Genotypes**	**OR (95% CI)**	***P*-value**	**OR (95%CI)**	***P*-value**	**OR (95%CI)**	***P*-value**
rs11646411	CDH13	G-[b]	1	—	1	—	1	—
		CC	0.968 (0.604–1.550)	0.892	1.465 (0.858–2.502)	0.162	0.938 (0.660–1.334)	0.723
rs6565113	CDH13	TT	1	—	1	—	1	—
		GT	0.903 (0.571–1.428)	0.663	1.018 (0.641–1.615)	0.940	1.188 (0.843–1.674)	0.325
		GG	0.872 (0.502–1.515)	0.626	0.892 (0.466–1.706)	0.730	0.785 (0.521–1.184)	0.248
rs11150556	CDH13	T-[c]	1	—	1	—	1	—
		CC	1001 (0.648–1.545)	0.999	1.601 (1.040–2.465)	0.096	1.032 (0.744–1.431)	0.999
rs13395022	CTNNA2	C-[b]	1	—	1	—	1	—
		TT	1.185 (0.788–1.782)	0.415	1.098 (0.720–1.674)	0.664	1.260 (0.935–1.697)	0.128

SNP, Single Nucleotide Polymorphism; OR, Odds Ratio; CI, Confidence Interval.
[a]Multivariate logistic regression adjusted for gender and age.
[b]The homozygous for the minor allele and the heterozygous genotypes were polled due to low frequency.
[c]T carriers were pooled with CC homozygous because they were previously associated with worse performance in VWM task; *P*-values were corrected by false discovery rate.

TABLE III. Mean Hyperactivity/Impulsivity Symptoms According to *rs11150556 CDH13* Genotypes Across the Three Groups

Sample	*rs11150556 genotypes*	N	Mean	± SE	95% IC Lower bound	95% IC Upper bound	F	*P*-value	FDR[e]
Children/adolescents from ProDAH[a]	TT	52	1.521	0.127	1.271	1.771			
	CT	144	1.443	0.087	1.273	1.614	4.579	0.011	0.026
	CC	80	1.771	0.104	1.567	1.976			
	T_[d]	196	1.461	0.082	1.300	1.622	8.800	0.003	0.018
	CC	80	1.768	0.104	1.564	1.972			
1993 Pelotas birth cohort[b]	TT	22	4.10	0.426	3.25	4.94			
	CT	46	4.74	0.305	4.13	5.34	3.969	0.022	0.033
	CC	42	5.54	0.320	4.90	6.17			
	T_[d]	68	4.52	0.250	4.02	5.02	6.400	0.013	0.026
	CC	42	5.53	0.321	4.89	6.16			
Adults from ProDAH[c]	TT	108	1.518	0.071	1.378	1.658			
	CT	243	1.518	0.048	1.424	1.612	0.370	0.691	0.691
	CC	131	1.453	0.065	1.326	1.580			
	T_[d]	351	1.518	0.040	1.440	1.596	0.742	0.389	0.456
	CC	131	1.453	0.065	1.326	1.580			

[a]Hyperactive/Impulsive SNAP-IV scores was the dependent variable; ANOVA was adjusted for gender and ethnicity.
[b]The dependent variable in the models was DSM-V hyperactivity/impulsivity symptoms; ANOVA was adjusted for ethnicity.
[c]Hyperactive/Impulsive SNAP-IV scores was the dependent variable; ANOVA was performed with adjustment for gender.
[d]*TT* + *CT* against the CC genotype.
[e]*P*-values adjusted for false discovery rate correction.

from childhood [Shaw et al., 2013]. Comparing to typically developing subjects, persistent ADHD patients showed a fixed deficit in cortical maturation whereas remitted ADHD individuals converted to normal maturation pattern. Moreover, the link between cortical trajectories and ADHD outcome seems to be driven by inattentive symptoms [Shaw et al., 2013].

The majority of Cadherin-13 expression studies are only suggestive regarding the involvement of this atypical cadherin in the regulation of brain development and function. Several experimental findings indicate that Cadherin-13 expression is not restricted to a specific cell type suggesting a key role for Cadherin-13 in several basic functions of the developing and adult brain [Rivero et al., 2013]. Moreover, Cadherin-13 expression pattern overlaps with regions showing volumetric reductions in patients with ADHD, such as the cerebellum and pre-frontal cortex [Valera et al., 2007]; [Konrad and Eickhoff, 2010].

During mammalian brain development, monoaminergic terminals coming from the dopaminergic *substantia nigra* and ventral tegmental area, the noradrenergic *locus coeruleus* or the serotonergic dorsal raphe will be guided to their peripheral prefrontal cortical targets by different membrane bound or soluble proteins that act as guidance molecules. One of these guidance molecules would be Cadherin-13, with a critical function as a signaling molecule rather than a typical adhesion molecule [Riveros et al, 2013]. Although different lines of evidence suggest Cadherin-13 to be a negative regulator of neurite extension and subsequent axonal pathfinding, the precise mechanism of Cadherin-13 action is unknown, and the role of Cadherin-13 in synaptogenesis remains elusive. Cadherin-13 expression appears to be higher in the adult than in the developing brain, at least in humans [Takeuchi et al., 2000]. Therefore a certain versatility of Cadherin-13 throughout the lifespan seems most likely, first as an axonal pathfinder during neurodevelopment, and, once the neuronal circuits have been established, it may be more relevant for the maintenance of synapses and/or the coordination of processes critical for synaptic plasticity [Riveros et al., 2013].

Symptomatic threshold for diagnosing ADHD is a controversial issue and the disorder seems to be better understood at the dimensional level [Larsson et al., 2012; Salum et al., 2014]. A quantitative view of ADHD could facilitate etiologic investigations, considering that genetic and environmental factors seem to operate dimensionally throughout the distribution of ADHD symptoms [Larsson et al., 2012]. Moreover, genetic studies of symptom domains might increase the strength of association analysis due to a decrease in phenotypic heterogeneity [Bralten et al., 2013]. Therefore, in addition to genetic association analyzes by case-control strategies, quantitative analyses should be performed in genetic studies of some complex phenotypes, such ADHD, when it is possible or plausible [Lewis and Knight, 2012].

Considering the decrease in hyperactive/impulsive symptoms over the life cycle, association analysis of this symptom domain with neurodevelopmental genes at different age ranges seems to be relevant to understand ADHD [Bralten et al., 2013]. Moreover, genetic investigations of symptom domains separately might help

in genetic risk variants identification of specific dimensions [Bralten et al., 2013]. In both childhood and adulthood, a 60–70% genetic correlation between hyperactivity-impulsivity and inattention has been estimated in ADHD patients [Franke et al., 2012]. Therefore, even considering that these two symptom domains compose ADHD diagnosis and share a proportion of genetic effects, there is an important genetic specificity for each symptom group [Greven et al., 2011].

This work should be viewed in light of certain limitations. First, in this study there is a disproportion between cases and controls in the children/adolescent and cohort samples. Ideally, a similar number of case and control subjects would be expected. Indeed, this disproportion between case and control subsamples tends to increase the probability of a false positive finding. However, the results of case-control analyses were negative [Balding, 2006]. Second, this is a cross-sectional study whereas it would be better to investigate the involvement of neurodevelopmental genes in a longitudinal approach. Third, although we included ethnicity as a covariate in the ANOVA analyses, no genomic control was performed. Therefore, our findings could have been biased by hidden genetic heterogeneity present in this sample. Putting all together, these evidences suggest a putative role of *CDH13* gene underlying hyperactive/impulsive symptoms of ADHD during development. Although the effect sizes were small, as would be expected for single genes in multifactorial diseases, our results showed that the involvement of *CDH13* in hyperactive/impulsive domain in ADHD seems to be more evident in childhood and adolescence than in the adulthood.

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