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BRIEF COMMUNICATION

Sequence analysis of the *ADRA2A* coding region in children affected by attention deficit hyperactivity disorder

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Abstract Attention deficit hyperactivity disorder (ADHD) is a common neurobehavioral pathology characterized by distinct degrees of inattention, hyperactivity and impulsivity. Although ADHD etiology remains elusive, the *ADRA2A* candidate gene underlies a particular interest, since it participates in the prefrontal cortex regulation of executive function. Three SNPs located on 5′ and 3′UTR regions of the gene have been extensively explored but none of them have been definitely validated as a predisposition or a causative sequence variation. In this study, in order to determine whether *ADRA2A* non-synonymous sequence variants, resulting in biochemical modifications of the protein, are a common cause of the disease we sequenced the complete *ADRA2A* coding region in a panel of ADHD children of Colombian origin. We identified the c.1138 C>A (p.Arg380Arg) silent substitution. We conclude that *ADRA2A* non-synonymous sequence variants do not cause ADHD in our sample population. We cannot formerly discard a potential role of this gene during ADHD pathogenesis since only the coding region was analysed. We hope that these results will encourage further researchers to sequence the promoter and coding regions of *ADRA2A* in large panels of ADHD patients from distinct ethnical origins.

Keywords Attention deficit hyperactivity disorder · ADHD · *ADRA2A* · Genetics · Behavior

Introduction

Attention deficit hyperactivity disorder (ADHD) is a common neurobehavioral pathology characterized by distinct degrees of inattention, hyperactivity and impulsivity, which appear during childhood and lead to different types of impairment. Despite advances in the diagnosis and treatment of behavior disorders, ADHD etiology remains poorly understood. Candidate gene approaches led to propose molecular markers within relevant genes, such as *DRD4, DRD5*, *HTR1B*, *SLC6A4*, *SNAP-25*, *DBH* and *SLC6A* [1, 2]. Although several reports proposed genetic associations of SNPs located within further pertinent candidate genes (e.g. *DRD2*, *HTR1B*, *COMT*, *CHRNA4*, *GRIN2A*, *ADRA2A*), they frequently displayed discordant results due to the phenotypic and genetic complexities of the disease [1, 3, 4].

Among ADHD candidate genes *ADRA2A* underlies a particular interest, since it participates in the prefrontal cortex (PFC) regulation of executive functions and emotion [5]. The noradrenaline stimulation of ADRA2A receptors, located on the spines of PFC pyramidal cells, contributes to strengthen synaptic connections by closing ion channels

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responsible to modulate the synaptic inputs. Up to now, three SNPs (rs1800544, rs1800545, rs553668) located on 5′ and 3′UTR regions of the gene have been extensively explored by linkage and association studies but none of them have been definitely validated as a predisposition or a causative sequence variation [6].

ADHD, as well as other behavioral phenotypes, might originate from small to moderate additive effects conferred by genomic variants located on coding and regulatory regions of many genes. Similarly to other common pathologies, ADHD might also be caused by effects of non-synonymous variants present in a small repertoire of key genes [7]. The identification of such mutations underlines a particular interest as it would permit a better understanding of molecular pathways related to physiological and pathological traits. Therefore, we hypothesize that non-synonymous *ADRA2A* sequence variants, resulting in biochemical modifications of the protein, might cause ADHD.

Patients and methods

Eighty-four ADHD children were recruited from low and medium socioeconomic public and private schools of Bogotá, Colombia. Clinical screening was performed following DSM IV-ADHD criteria. Experienced neurologists and psychiatrists reviewed all clinical diagnosis. To confirm ADHD, we performed in teachers and patients′ parents, the Behavior Assessment System for Children Scale (BASC). The Revised Wechsler Intelligence Scale for Children (WISC-R) was used to exclude children with cognitive deficits: children with score below 70 were not taken into account for the study. This methodology allowed us to identify 31 children belonging to the inattentive type, 11 to the hyperactive-impulsive type and 42 to the combined type (Table 1). Clinical examinations include gender, age and full IQ scale classification (Table 1). Individuals with parents′ reports of other neurological impairment were excluded from the study. Parents provided a written informed consent on behalf of their children. Clinical and experimental steps of this study were approved by the School of Medicine Ethics Committee (El Rosario University).

Genomic DNA from patients was obtained from whole blood samples using the phenol–chloroform procedure. The complete coding region of the gene was amplified by PCR using exon-flanking primers. *ADRA2A* open reading frame was sequenced with internal primers. Sequences were compared to that of the *ADRA2A* wild type version (ENSG00000150594). NCBI and Ensembl databases of SNPs were used to identify previously reported sequence variants.

Table 1 Demographic and clinical characteristics of ADHD patients

	ADHD patients
Number	84
Mean age	8.7 (SD = 2.6)
Female	16 (19 %)
Male	68 (81 %)
Full IQ scale	92.2 (SD = 14.9)
Inattentive type	31 (36.9 %)
Hyperactive-impulsive type	11 (13.1 %)
Combined type	42 (50 %)

SD standard deviation

Results and discussion

Sequencing analysis of the complete *ADRA2A* coding region revealed the synonymous c.1138 C>A (p.Arg380Arg) variant. This nucleotide change, which was present at homozygous and heterozygous states, was previously reported in public databases of SNPs (rs1800038) (Table 2). We did not find further sequence variants.

The potential association between *ADRA2A* sequence variations and ADHD etiology was explored for the first time by Xu and co-workers [8]. In this study the -1291C>G SNP (rs1800544) was genotyped in ADHD patients and non-affected relatives but no association was encountered. Since then, further studies on this and other *ADRA2A* specific SNPs (e.g. rs1800545, rs553668) have been performed [5, 9–17]. Unfortunately, they did not permit to formerly associate them to the phenotype, as in some cases they displayed contradictory results. Some studies identified an association of the *ADRA2A -1291 C>G* sequence variant with the phenotype. For instance some of them

Table 2 Allelic and genotypic frequencies of the *ADRA2A* c.1138 C>A sequence variant identified in Colombian ADHD patients

	AF[a]		GF[b]		
	C	A	CC	CA	AA
Total ADHD ($n = 84$)	0.88	0.12	65 (77.4 %)	18 (21.4 %)	1 (1.2 %)
Inattentive ($n = 31$)	0.85	0.15	22 (70.9 %)	9 (29.1 %)	0
Hyperactive-impulsive ($n = 11$)	0.90	0.10	9 (81.8 %)	2 (18.2 %)	0
Combined ($n = 42$)	0.89	0.11	34 (80.9 %)	7 (16.7 %)	1 (2.4 %)

[a] Allelic frequency

[b] Genotypic frequency

described an association between this SNP and the inattentive symptoms observed in ADHD individuals [18, 19]. Other report, described an association with both hyperactive and inattentive symptoms [10, 20]. Finally, further studies did not show an association with the disease [1]. This scenario suggests that assortments of sequence variants (haplotypes) located on regulatory and/or coding regions of the gene might contribute to the phenotype. On this point, Park et al. [10] suggested that the less common -1291G allele contributes to an increased risk to ADHD, especially when inattentive clinical features are considered. In addition, the potential role of this SNP has been related, by pharmacogenetics, with differential clinical responses to methylphenidate (MPH). Indeed, some reports show that the G allele improves the pharmacological response to this molecule [21–23].

In the present study, we hypothesized that nucleotide substitutions located on the *ADRA2A* coding region might alter the intrinsic physicochemical properties of the protein and, in fine, provoke and/or contribute to ADHD phenotype. From this point of view, we estimate that the synonymous nucleotide substitution described here is not related to the disease, since it does not modify the ADRA2A aminoacid sequence. However, we cannot formerly discard a potential role of this gene during ADHD pathogenesis since only the coding region was analysed. The sequence variant found in our study might constitute a part of an haplotype, which might include previously described or undiscovered sequence variants. Furthermore, although the present work represents the largest molecular study performed in Colombian ADHD patients, we estimate that the small sample size constitutes a limitation. We hope that these results will encourage further researchers to sequence the promoter and coding regions of *ADRA2A* in large panels of ADHD patients from distinct ethnical origins.

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Conflict of interest The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this research.

References

1. Faraone SV, Mick E (2010) Molecular genetics of attention deficit hyperactivity disorder. Psychiatr Clin North Am 33:159–180
2. Caylak E (2007) A review of association and linkage studies for genetical analyses of learning disorders. Am J Med Genet B Neuropsychiatr Genet 144:923–943
3. Gizer IR, Ficks C, Waldman ID (2009) Candidate gene studies of ADHD: a meta-analytic review. Hum Genet 126:51–90
4. Caylak E (2012) Biochemical and genetic analyses of childhood attention deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 159:613–627
5. Waldman ID, Nigg JT, Gizer IR, Park L, Rappley MD, Friderici K (2006) The adrenergic receptor alpha-2A gene (ADRA2A) and neuropsychological executive functions as putative endophenotypes for childhood ADHD. Cogn Affect Behav Neurosci 6:18–30
6. Banaschewski T, Becker K, Scherag S, Franke B, Coghill D (2010) Molecular genetics of attention-deficit/hyperactivity disorder: an overview. Eur Child Adolesc Psychiatry 19:237–257
7. Gibson G (2011) Rare and common variants: twenty arguments. Nat Rev Genet 13:135–145
8. Xu C, Schachar R, Tannock R et al (2001) Linkage study of the alpha2A adrenergic receptor in attention-deficit hyperactivity disorder families. Am J Med Genet 105:159–162
9. Roman T, Schmitz M, Polanczyk GV, Eizirik M, Rohde LA, Hutz MH (2002) Further evidence for the association between attention-deficit/hyperactivity disorder and the dopamine-beta-hydroxylase gene. Am J Med Genet 114:154–158
10. Park L, Nigg JT, Waldman ID et al (2005) Association and linkage of alpha-2A adrenergic receptor gene polymorphisms with childhood ADHD. Mol Psychiatry 10:572–580
11. Stevenson J, Langley K, Pay H et al (2005) Attention deficit hyperactivity disorder with reading disabilities: preliminary genetic findings on the involvement of the ADRA2A gene. J Child Psychol Psychiatry 46:1081–1088
12. Brookes K, Xu X, Chen W et al (2006) The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. Mol Psychiatry 11:934–953
13. Deupree JD, Smith SD, Kratochvil CJ et al (2006) Possible involvement of alpha-2A adrenergic receptors in attention deficit hyperactivity disorder: radioligand binding and polymorphism studies. Am J Med Genet B Neuropsychiatr Genet 141:877–884
14. Wang B, Wang Y, Zhou R et al (2006) Possible association of the alpha-2A adrenergic receptor gene (ADRA2A) with symptoms of attention-deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 141:130–134
15. Schmitz M, Denardin D, Silva TL et al (2006) Association between alpha-2a-adrenergic receptor gene and ADHD inattentive type. Biol Psychiatry 60:1028–1033
16. Cho SC, Kim JW, Kim BN et al (2008) Possible association of the alpha-2A-adrenergic receptor gene with response time variability in attention deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 147:957–963
17. Guan L, Wang B, Chen Y et al (2009) A high-density single-nucleotide polymorphism screen of 23 candidate genes in attention deficit hyperactivity disorder: suggesting multiple susceptibility genes among Chinese Han population. Mol Psychiatry 14:546–554
18. Roman T, Polanczyk GV, Zeni C, Genro JP, Rohde LA, Hutz MH (2006) Further evidence of the involvement of alpha-2A-adrenergic receptor gene (ADRA2A) in inattentive dimensional scores of attention-deficit/hyperactivity disorder. Mol Psychiatry 11:8–10
19. Schmitz M, Denardin D, Silva TL et al (2006) Association between alpha-2a-adrenergic receptor gene and ADHD inattentive type. Biol Psychiatry 60:1028–1033
20. Roman T, Schmitz M, Polanczyk GV, Eizirik M, Rohde LA, Hutz MH (2003) Is the alpha-2A adrenergic receptor gene (ADRA2A) associated with attention-deficit/hyperactivity disorder? Am J Med Genet B Neuropsychiatr Genet 120:116–120
21. Polanczyk G, Zeni C, Genro JP et al (2007) Association of the adrenergic alpha2A receptor gene with methylphenidate improvement of inattentive symptoms in children and adolescents

with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 64:218–224
22. da Silva TL, Pianca TG, Roman T et al (2008) Adrenergic alpha2A receptor gene and response to methylphenidate in attention-deficit/hyperactivity disorder-predominantly inattentive type. J Neural Transm 115:341–345
23. Cheon KA, Cho DY, Koo MS, Song DH, Namkoong K (2009) Association between homozygosity of a G allele of the alpha-2a-adrenergic receptor gene and methylphenidate response in Korean children and adolescents with attention-deficit/hyperactivity disorder. Biol Psychiatry 65:564–570