Genetics and Molecular Biology, 35, 4 (suppl), 932-938 (2012)

www.sbg.org.br

Review Article

The Brazilian contribution to Attention-Deficit/Hyperactivity Disorder molecular genetics in children and adolescents

Júlia Pasqualini Genro[1], Tatiana Roman[2], Luis Augusto Rohde[3] and Mara Helena Hutz[2]

[1]*Departamento de Ciências Básicas da Saúde, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil.*
[2]*Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.*
[3]*Divisão de Psiquiatria da Infância e Adolescencia, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, and Instituto de Psiquiatria do Desenvolvimento para a Infância e Adolescência, Porto Alegre, Brazil.*

Abstract

Attention-Deficit/Hyperactivity Disorder (ADHD) is a common psychiatric condition of children worldwide. This disorder is defined by a combination of symptoms of inattention and hyperactivity/impulsivity. Diagnosis is based on a sufficient number of symptoms causing impairment in these two domains determining several problems in personal and academic life. Although genetic and environmental factors are important in ADHD etiology, how these factors influence the brain and consequently behavior is still under debate. It seems to be consensus that a fronto-subcortical dysfunction is responsible, at least in part, for the ADHD phenotype spectrum. The main results from association and pharmacogenetic studies performed in Brazil are discussed. The investigations performed so far on ADHD genetics in Brazil and elsewhere are far from conclusive. New plausible biological hypotheses linked to neurotransmission and neurodevelopment, as well as new analytic approaches are needed to fully disclose the genetic component of the disorder.

Keywords: ADHD, candidate genes, pharmacogenetics.

Attention-deficit/hyperactivity disorder (ADHD) is a common psychiatric condition affecting approximately 5.3% of children and adolescents worldwide. Although some authors have described ADHD as an American disorder, a meta-analysis showed that the variation in prevalence rates worldwide is more related to methodological differences among studies than to geographic localization and consequently cultural factors (Polanczyk *et al*., 2007a).

Rohde *et al*. (2005) reviewed seven studies reporting prevalence rates of ADHD in Brazil and reported a high variability of this disorder (1.5% to 18%), which was attributed to the use of completely diverse methodologies. One of these studies was performed with a representative sample of 1013 randomly selected students from public schools in Porto Alegre and reported an ADHD prevalence of 5.8% using full DSM-IV criteria (Rohde *et al*. 1999). In a more recent investigation, Polanczyk *et al*., (2010) assessed a representative household sample of the Brazilian population. The sample comprised 3007 individuals, and the overall prevalence of positive screeners for ADHD was 5.8% [95% confidence interval (CI), 4.8-7.0].

Send correspondence to Mara H. Hutz. Departamento de Genética, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, 91501-970 Porto Alegre, RS, Brazil. E-mail: mara.hutz@ufrgs.br.

Developmentally inappropriate and impairing levels of symptoms in two behavior dimensions -in attention and hyperactivity-impulsivity - characterize ADHD. As currently recognized by the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV), these dimensions may be expressed at different extents among children with ADHD defining three clinical subtypes: primarily inattentive, primarily hyperactive/impulsive, and combined subtypes of the disorder (American Psychiatric Association, 1994).

The ratio of boys to girls with ADHD is between 3:1 and 9:1 in clinical samples. Part of the gender difference may be due to referral bias related to symptoms of disruptive behavior, since boys have both more hyperactive/impulsive and conduct/oppositional symptoms than girls. In Brazilian clinical samples, similar ratios have been observed (Roman *et al*, 2001; Rohde *et al*. 2005). Approximately 65% of children with the diagnosis continue to show symptoms of ADHD in adulthood, suggesting that ADHD

could be considered a chronic developmental disorder (Faraone *et al*., 2006).

Attention-deficit/hyperactivity disorder is a complex and heterogeneous disorder and its etiology is not yet completely understood (Genro *et al*., 2010). Despite evidence that environmental factors play an important role in its etiology, classical genetics studies support a strong genetic contribution for ADHD development. The risk of ADHD among parents of children with ADHD is increased by 2 to 8 fold comparing with the population rate (Faraone *et al*., 2005). A meta-analysis with 20 pooled twin studies estimated an average heritability of 76% suggesting that ADHD is one of disorders with the strongest genetic component in psychiatry (Faraone *et al*., 2005).

Neurobiological studies suggests ADHD as a frontal-striatum-cerebellum disease (Curatolo *et al*., 2009; Makris *et al*., 2009), since these regions present lower volume and activity in patients with this disorder. Such observations are corroborated by neuropsychological data, which show that ADHD children have a poorer performance in cognitive and executive functions and behavioral inhibition failures, neuropsychological processes clearly related to frontal lobe and sub-cortical areas (Makris *et al*., 2009).

The dopamine (DA) system has been the main focus to explain the disorder. The dopaminergic theory, proposed by Levy in 1991, suggested that DA deficits in specific brain regions, such as cortical areas and the striatum, could produce ADHD symptoms (Levy, 1991). It seems to be a consensus in the literature that a fronto-subcortical dysfunction is responsible for at least part of the ADHD spectrum (Genro *et al*., 2010). Recently other systems, like the serotoninergic and glutamatergic one have been suggested to have a role in ADHD etiology (Curatolo *et al*., 2009).

The dopaminergic theory proposed to explain the neurobiology of the disorder led to the first association study by Cook *et al*. (1995). These authors investigated a 40 bp variable number of tandem repeats (VNTR) located in the 3' untranslated region of the dopamine transporter gene (*DAT1*) in ADHD families. Using the family-based approach haplotype relative risk (HRR), an association with the 10-repeat allele (10R) was detected. In the following year, LaHoste *et al.* (1996) investigated another dopaminergic gene, the dopamine D4 receptor gene (*DRD4*). In that study, the frequency of a 48 bp VNTR located in exon 3 was compared between ADHD cases and controls, and an association with the 7-repeat allele (7R) was observed. These two genes, specifically through these variants, became the most studied genes in ADHD molecular genetics, with either positive or negative results.

Based on neurobiological hypotheses, genes from different neurotransmission systems were investigated. Both case-control and family-based methods were used, as well as different approaches to define ADHD phenotype. Although the results are still controversial, as discussed later, some genes have been suggested as susceptibility genes; besides *DAT1* and *DRD4*, the genes encoding dopamine D5 receptor (*DRD5*), dopamine beta-hydroxylase (*DBH*), serotonin 1B receptor (*5HTR1B*), serotonin transporter (*5HTT*), synaptossomal-associated protein of 25 kDa (SNAP25) have shown positive findings in different samples (Mick and Faraone, 2008). However, even for these genes only a very small effect has been detected in most studies (Gizer *et al*., 2009). For example, an odds ratio of 1.33 was demonstrated for the *DRD4* gene 7R allele, while for the *DAT1* 10R allele the pooled odds ratio was 1.12. This study also evidenced a marked heterogeneity in the magnitude of association reported in the different studies, which seems to vary largely according to the analyses used, among other reasons. Independent of detected effect sizes, the overall findings strongly suggest that each gene (or variant) can explain just a tiny part of the ADHD phenotypic variance, and that no one can be considered sufficient or necessary to cause the disease.

ADHD genetics in Brazil began just a few years after the publication by Cook *et al*. (1995). In 1998, the ADHD Outpatient Clinic (PRODAH) was created by one of us (LAR) linked to Hospital de Clínicas de Porto Alegre, the teaching-hospital of our University. The PRODAH soon became a reference center for ADHD diagnosis and treatment, also stimulating the development of different research areas, including genetics. It is important to note that, although we will focus this paper on our findings from genetics in children and adolescents, other investigators in our program are dedicated to studying genetics in adults with ADHD. Some reports on ADHD genetics from other Brazilian labs can also be found. The first paper published by our group was in 2001. In that report, Roman *et al*. (2001) investigated both *DAT1* and *DRD4* genes, using case-control, family-based and dimensional approaches. Case-control analysis showed an association of ADHD with the *DRD4* 7R allele. However, this finding was subject to criticism since the controls were adults ascertained at a paternity testing service. The family-based analysis showed no association for both genes. However, through the dimensional approach a very interesting result was observed. The number of inattentive and hyperactive/impulsive symptoms among patients according to the presence of risk genotypes (*i.e.*, presence of 7R *vs.* other *DRD4* genotypes and 10R/10R homozygous *vs.* other *DAT1* genotypes) was compared,showing an interaction effect: the presence of both risk conditions was related to a significantly higher number of hyperactive/impulsive symptoms. This paper was important not only because it was the first genetic report from our ADHD research group, but also because it was the first to investigate the possibility of gene-gene interaction, a situation unexplored in molecular studies so far and even today not extensive explored in the ADHD arena.

A summary of our association studies is presented in Table 1. These reports were focused on genes from dopaminergic, noradrenergic and serotonergic systems, and sev-

eral positive results were observed, some of these related to specific aspects of the disorder. For example, an interesting result was obtained for the serotonin 2A receptor gene, *5HTR2A*, where the family-based analyses showed association only in the group of male patients. One of the most important findings, however, are those related to alpha 2A adrenergic receptor gene *ADRA2A* that have consistently suggested an association between the -1291C > G polymorphism (*Msp*I, rs1800544) specifically with inattentive symptoms in our population. Furthermore, Schmitz *et al*. (2006) reported an association between this same genotype in an independent sample composed only by the inattentive subtype.

ADHD pharmacogenetics has also been the focus of several investigations (Table 2). Roman *et al*. (2002b) produced the second paper on ADHD pharmacogenetics in the literature, replicating previous findings from Winsberg and Comings (1999) that suggested an influence of *DAT1* 3' VNTR on MPH response, but this finding was not observed in an independent sample from the same population (Zeni *et al*., 2007). The *ADRA2A* findings suggest a role for -1291C > G SNP in the improvement of inattentive symp-

Table 1 - Association studies conducted in Brazilian children and adolescents.

Gene	Study design	Main results	References
Dopaminergic system			
DRD4/DAT1	Population-based case-control, family-based and quantitative analyses	Association with *DRD4* 7R allele by case-control analysis. No association with either *DRD4* or *DAT1* by family-based approach. Significantly higher number of hyperactive/impulsive symptoms in the presence of 7R allele at *DRD4* and 10R/10R homozygosity at *DAT1*	Roman *et al*., 2001
DRD5	Family-based analysis (multicenter study with inclusion of a Brazilian sub-sample)	Association with the 148-bp allele of the (CA)(n) repeat polymorphism, located 18.5 kb from the *DRD5* gene; association confined to the predominantly inattentive and combined clinical subtypes	Lowe *et al*., 2004
DAT1	Family-based analysis	Biased transmission of the C allele at -839 C > T polymorphism (rs 2652511) in total ADHD sample, strengthened when the analyses were restricted to the ADHD combined type. No association with the 3' VNTR	Genro *et al*., 2007
DAT1	Family-based analysis	Preferential transmission of the haplotype A/C/C/C/A derived respectively from SNPs rs 2550948, rs 11564750, rs 261759, rs 2652511 and rs 2975223 in 5' region and no association with any allele/haplotype at the 3' region of the gene, including the 3' VNTR and the VNTR of intron 8	Genro *et al*., 2008
Noradrenergic system			
DBH	Family-based analysis	Preferential transmission of the *Taq*I (rs 129914) A2 allele in the whole ADHD sample, strengthened when the analysis was restricted to families with no ADHD parental diagnosis	Roman *et al*., 2002a
ADRA2A	Family-based and quantitative analysis	No association with either *Msp*I allele through the family-based analysis, but increased inattention and combined (hyperactivity/impulsivity + inattention) symptom scores in the presence of GG genotype	Roman *et al*., 2003
ADRA2A	Quantitative analysis	Significantly higher inattention symptom scores in the presence of GG genotype at *Msp*I polymorphism	Roman *et al*., 2006
ADRA2A	Population-based case-control analysis	Significantly higher odds ratio for ADHD-I[#] in homozygous patients for the *Msp*I G allele	Schmitz *et al*., 2006
Serotonergic system			
5HTT	Family-based analysis	No evidence for biased transmission of any allele at the repeat polymorphism in the promoter region (5-HTTLPR[#]), both for the whole sample and for male probands only	Guimarães *et al*., 2007
5HTR2A	Family-based analysis	No evidence for biased transmission of any allele at the -1438A > G SNP (rs 6311), both for the whole sample and for male probands only. Preferential transmission of the His452 allele at His452Tyr SNP (rs 6314), only in families with male probands	Guimarães *et al*., 2007
5HTR1B	Family-based analysis	Biased transmission of haplotype G/T/G derived respectively from SNPs -261T > G (rs 11568817), -161A > T (rs 130058), 861G > C (rs 6296) in the total sample	Guimarães *et al*., 2009a

[#]ADHD-I: ADHD inattentive subtype; 5-HTTLPR: serotonin transporter gene-linked polymorphic region.

Table 2 - Pharmacogenetic studies performed in Brazilian children and adolescents.

Gene	Approach	Main results	References
Dopaminergic system			
DAT1	ADHD boys treated with MPH, efficacy of the medication measured by scores obtained from ABRS[#] and CGAS[#] scales	Patients with 10R/10R genotype demonstrated significantly worse response to MPH according to both ABRS and CGAS scores	Roman *et al*., 2002b
DAT1/*DRD4*	ADHD patients treated with MPH, outcome measures by SNAP IV[#], CGAS and SERS[#], assessed at baseline and 1 month after the intervention	No significant association was detected between polymorphisms studied (*DRD4* exon 3 VNTR and *DAT1* 3' VNTR) and both response and side effects to MPH	Zeni *et al*., 2007
Noradrenergic system			
ADRA2A	ADHD patients treated with MPH, outcome measures by SNAP IV and SERS, assessed at baseline and 1 and 3 months after the intervention	A significant interaction effect between the presence of *Msp*I G allele and treatment with MPH over time on inattentive scores during the 3 months of treatment	Polanczyk *et al*., 2007b
ADRA2A	ADHD-I[#] patients treated with MPH, outcome measures by inattentive subscale of SNAP IV, assessed at baseline and 1 month after the intervention	Significantly lower inattentive scores with MPH treatment at the first month of treatment in patients with *Msp*I G allele	Da Silva *et al*., 2008
Serotonergic system			
5HTT, *5HTR2A*, *5HTR1B*	ADHD patients treated with MPH, outcome measures by SNAP IV, CGAS and SERS, assessed at baseline and 1 month after the intervention	No significant association was detected between polymorphisms assessed (5-HTTLPR[#], *5HTR1B* 861G > C, *5HTR2A* His452Tyr) and both response and side effects to MPH	Zeni *et al*., 2007
Metabolic enzymes			
MAOA[#]	ADHD patients treated with MPH, outcome measures by oppositional subscale of SNAP IV, assessed at baseline and 1 and 3 months after the intervention	A significant interaction effect between the presence of MAOA high-activity genotype at promoter region uVNTR[#] and treatment with MPH over time on oppositional scores during the 3 months of treatment	Guimarães *et al*., 2009b
COMT	ADHD patients treated with MPH, outcome measures by oppositional subscale of SNAP IV, assessed at baseline and 1 and 3 months after the intervention	A significant effect of the presence of Met allele at Val158Met (rs 4680) in oppositional defiant disorder scores during treatment and a significant interaction between the Met allele and treatment over time for the SNAP-IV oppositional scores during this period of treatment	Salatino-Oliveira *et al*., 2011

[#] *MAOA*: monoamino oxidase A gene; ABRS: Conners Abbreviated Rating Scale; CGAS: Children's Global Assessment Scale; SNPA-IV: Swanson, Nolan, and Pelham scale-version IV; SERS: Barkley's Stimulants Side Effects Rating Scale; ADHD-I: ADHD inattentive subtype; 5-HTTLPR: serotonin transporter gene-linked polymorphic region; uVNTR: VNTR at the promoter region of *MAOA* gene.

toms, which concur with the association studies that emphasized the influence of the *ADRA2A* gene on ADHD. Guimarães *et al*. (2009b) and Salatino-Oliveira *et al*. (2011) reported an effect of MPH treatment on oppositional symptoms in ADHD patients. Table 3 shows a timeline of our most relevant publications, as well as seminal worldwide papers, so as to contextualize our results within the worldwide literature.

The relation between genetic and possible neurobiological markers was also assessed. In the work by Rohde *et al*. (2003), ADHD boys treated with MPH were compare, according to the regional cerebral blood flow (rCBF), during an attention test (Continuous Performance Test, CPT), using single photon emission computerized tomography (SPECT) to assess neuroimaging measures. A significantly higher rCBF in medial frontal and left basal ganglia areas in children homozygous for the *DAT1* 10R allele was observed, suggesting that a higher dopamine activity is needed to achieve a response to MPH in these patients. In a neuroimaging/genetic study, a significantly higher perfusion in the right middle temporal gyrus (MTG) in patients with both 7R allele at *DRD4* and 10R homozygosity at *DAT1* was also verified (Szobot *et al*., 2005). MTG is a region related to functions usually altered in ADHD patients. In this study, rCBF was also compared through SPECT among ADHD boys performing a CPT test. It is important to note that this was one of the first studies in the ADHD arena of what is now called genetics neuroimaging. Recently, a *DRD4* and *DAT1* interaction was once again evidenced. ADHD patients with comorbid substance use disorders were submitted to a SPECT scan with [Tc(99m)]TRODAT-1 at baseline and after three weeks on

Table 3 - Timeline of international literature's and our group's most relevant publications.

Publications worldwide	Year	Publications PRODAH
Cook *et al.*1995: First genetics association study with *DAT1*	1995	
La Hoste *et al*., 1996: association study with *DRD4*	1996	
	1998	PRODAH begin
	2001	Roman *et al*., 2001: first genetics study with *DAT1* and *DRD4*
	2002	Roman *et al,* 2002: First pharmacogenetics study, association with *DAT1* and MPH response
Brookes *et al*., 2006: International Multi-centre ADHD Gene project (IMAGE) analyse 51 genes and report association with *DAT1*, *DRD4* and 16 other	2006	Roman *et al*., 2006: Association between *ADRA2A* and symptom scores
	2007	Polanczyk *et al*., 2007: Association between *ADRA2A* and MPH treatment
	2007	Genro *et al*., 2007: Association with *DAT1* promoter region
Faraone *et al*., 2008: Linkage analysis of ADHD with no significant results	2008	Genro *et al*., 2008: describes *DAT1* structure in our population and confirms the association with the promoter region
Gizer *et al.*, 2009: meta-analytic review confirming associations with several candidate genes and high heterogeneity between the studies	2009	Guimarães *et al*., 2009: Association of *MAOA* with MPH treatment
	2011	Salatino-Oliveira *et al*., 2011 : Association between *COMT* and MPH response with oppositional scores
	2012	Tovo-Rodrigues *et al*., 2012: association between *DRD4* rare variants and ADHD

MPH treatment, measuring caudate and putamen DAT binding potential. The presence of both 7R allele at *DRD4* and 10R homozygosity at *DAT1* was significantly associated with a reduced DAT occupancy by MPH in both regions, indicating a possible individual variability in treatment response (Szobot *et al*., 2011).

The relation between neuropsychological tests and genetics was also investigated. Kieling *et al*. (2006) compared ADHD patients according to CPT performance. The presence of 7R allele at *DRD4* was associated with more errors, while 4R allele homozygosity was related to fewer errors. The performance in both CPT and a test of cognitive flexibility, the Wisconsin Card Sorting Test (WCST), was further investigated according to -1021 C > T genotypes at *DBH* gene; the CC homozygosity was associated with a diminished global performance in the tests (Kieling *et al*., 2008).

It has been extensively discussed in the literature (Mick and Faraone, 2008) that the associations found between several genes and ADHD are small and they are consistent with the *i.e.* that genetic vulnerability to ADHD is mediated by many genes of small effect. This scenario emphasizes the need for implementing strategies that can provide sufficient statistical power to detect such small effects.

One possible strategy to solve divergence in results is to investigate the whole gene structure and its variations. Most of the authors attribute the inconsistencies in association studies based on results from single markers as a consequence of variable linkage disequilibrium with a functional variant. However, to confirm this *i.e.* it would be essential to understand the linkage disequilibrium architecture in each studied population in an attempt to choose better markers and better interpret the results. This approach was used to investigate the DAT1 gene. Most association studies focused on the 3' UTR polymorphism and around half of them did not find any association, including our first study with this gene in 2001 (Roman *et al*., 2001). In an extended sample, we screened 16 polymorphisms across the gene to understand LD structure in the Brazilian sample (Genro *et al*., 2008). We replicated our negative result for the 3'UTR polymorphism, but we found a strong association with the promoter region of the gene. The structure of the gene in our population comprises 3 haplotype blocks and was similar with other LD results from different populations that described association with the 3' variant. Therefore, the differences between findings could not solely be explained by differences in LD architecture. For that reason, the most likely explanation for the inconsistencies regarding the role of the DAT1 gene in ADHD is that a combination of susceptibility variants across the gene exists and that these combinations differ among distinct populations. Our results strongly suggest that allele heterogeneity should be given due attention. This allele heterogeneity is commonly seen in several single gene disorders, but it is not generally considered in complex phenotypes or in candidate gene analyses. This strongly suggests that simply genotyping one marker per gene will provide little conclusive evidence for the association of that gene with a certain disease.

The role of rare variants rather than common variants in common diseases has been addressed in several recent investigations (Dickson *et al*, 2010). "Synthetic" signals of association could emerge due to a large proportion of genomic regions that harbor one or more rare variants that contribute to disease. An excess of rare variants were observed in the 7R alleles of ADHD patient when compared with controls. Furthermore, nucleotide changes that predict synonymous and non-synonymous substitutions were more common in the 7R sample (Tovo-Rodrigues *et al*., 2012). These findings suggest that not only repeat length but also DNA sequence should be assessed to better understand the role of the DRD4 exon 3 VNTR in ADHD genetic susceptibility. Taking together, these results strongly suggest that genetic heterogeneity must be considered in association studies.

In summary, the investigations performed so far in ADHD are far from conclusive. Most of the variants studied are located in non-coding regions of the human genome. Over the last decade, several studies have been performed to understand the functional role of common polymorphisms in the human genome. However, the methodology employed to investigate the relationships between gene expression and DNA variants is not fully established, and the results obtained so far have been controversial. Genes related to other neurotransmission and cell-cell communication systems are suggested in the literature, including processes such as cell division, adhesion and polarity, neuronal migration and plasticity, extracellular matrix regulation and cytoskeletal remodeling processes that indicate a whole range of new and promising possibilities for ADHD molecular genetic studies. Moreover, the candidate gene approach may not be the unique strategy for detecting susceptibility genes in complex diseases. Re-sequencing and *in silico* analyses might help to find new targets worthy of study. The knowledge of such genes might allow us to identify specific diagnostic biological markers. In addition, defining the target genes is the first step in developing novel drug therapies for ADHD.

Acknowledgments

Thanks are due to all our collaborators and students who obtained the data included in this review. The authors would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil), and Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul e de São Paulo (FAPERGS and FAPESP) for financial support.

References

American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders. 4th edition. American Psychiatric Association, Washington D.C., 943 pp.

Brookes K, Xu X, Chen W, Zhou K, Neale B, Lowe N, Anney R, Franke B, Gill M, Ebstein R, *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.

Cook EH, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE and Leventhal BL (1995) Association of attention deficit disorder and the dopamine transporter gene. Am J Hum Genet 56:993-998.

Curatolo P, Paloscia C, D'Agati E, Moavero R and Pasini A (2009) The neurobiology of attention deficit/hyperactivity disorder. Eur J Paediatr Neurol 13:299-304.

Da Silva TL, Pianca TG, Roman T, Hutz MH, Faraone SV, Schmitz M and Rohde LA (2008) Adrenergic alpha2A receptor gene and response to methylphenidate in attention-deficit/hyperactivity disorder-predominantly inattentive type. J Neural Transm 115:341-345.

Dickson SP, Wang K, Krantz I, Hakonarson H and Goldstein DB (2010) Rare variants create synthetic genome-wide associations. PLoS Biology 8:e1000294.

Faraone SV, Perlis RH, Doyle AE, Smooler JW, Goralnick JJ, Holmgren MA and Sklar P (2005) Molecular genetics of Attention-Deficit/Hyperactivity Disorder. Biol Psychiatry 57:1313-1323.

Faraone SV, Biederman J and Mick E (2006) The age-dependent decline of attention deficit hyperactivity disorder: A meta-analysis of follow-up studies. Psychol Med 36:159-165.

Faraone SV, Doyle AE, Lasky-Su J, Sklar PB, D'Angelo E, Gonzalez-Heydrich J, Kratochvil C, Mick E, Klein K, Rezac AJ, *et al.* (2008) Linkage analysis of attention deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 147B:1387-1391.

Genro JP, Zeni C, Polanczyk GV, Roman T, Rohde LA and Hutz MH (2007) A promoter polymorphism (-839 C > T) at the dopamine transporter gene is associated with attention deficit/hyperactivity disorder in Brazilian children. Am J Med Genet B Neuropsychiatr Genet 144B:215-219.

Genro JP, Polanczyk GV, Zeni C, Oliveira AS, Roman T, Rohde LA and Hutz MH (2008) A common haplotype at the dopamine transporter gene 5' region is associated with attention-deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 147B:1568-1575.

Genro JP, Kieling C, Rohde LA and Hutz MH (2010) Attention-deficit/hyperactivity disorder and the dopaminergic hypotheses. Expert Rev Neurother 10:587-601.

Gizer IR, Ficks C and Waldman ID (2009) Candidate gene studies of ADHD: A meta-analytic review. Hum Genet 126:51-90.

Guimarães AP, Zeni C, Polanczyk GV, Genro JP, Roman T, Rohde LA and Hutz MH (2007) Serotonin genes and attention deficit/hyperactivity disorder in a Brazilian sample: Preferential transmission of the HTR2A 452His allele to affected boys. Am J Med Genet B Neuropsychiatr Genet 144B:69-73.

Guimarães AP, Schmitz M, Polanczyk GV, Zeni C, Genro J, Roman T, Rohde LA and Hutz MH (2009a) Further evidence for the association between attention deficit/hyperactivity disorder and the serotonin receptor 1B gene. J Neural Transm 116:1675-1680.

Guimarães AP, Zeni C, Polanczyk G, Genro JP, Roman T, Rohde LA and Hutz MH (2009b) MAOA is associated with methylphenidate improvement of oppositional symptoms in boys with attention deficit hyperactivity disorder. Int J Neuropsychopharmacol 12:709-714.

Kieling C, Roman T, Doyle AE, Hutz MH and Rohde LA (2006) Association between *DRD4* gene and performance of children with ADHD in a test of sustained attention. Biol Psychiatry 60:1163-1165.

Kieling C, Genro JP, Hutz MH and Rohde LA (2008) The -1021 C/T DBH polymorphism is associated with neuropsychological performance among children and adolescents with ADHD. Am J Med Genet B Neuropsychiatr Genet 147B:485-490.

LaHoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N and Kennedy JL (1996) Dopamine D4 receptor gene polymorphism is associated with attention-deficit/hyperactivity disorder. Mol Psychiatry 1:121-124.

Levy F (1991) The dopamine theory of attention-deficit/hyperactivity disorder (ADHD). Aust N Z J Psychiatry 25:277-283.

Lowe N, Kirley A, Hawi Z, Sham P, Wickham H, Kratochvil CJ, Smith SD, Lee SY, Levy F, Kent L, *et al.* (2004) Joint analysis of the DRD5 marker concludes association with attention-deficit/hyperactivity disorder confined to the predominantly inattentive and combined subtypes. Am J Hum Genet 74:348-356.

Makris N, Biederman J, Monuteaux MC and Seidman LJ (2009) Towards conceptualizing a neural systems-based anatomy of attention-deficit/hyperactivity disorder. Dev Neurosci 31:36-49.

Mick E and Faraone SV (2008) Genetics of attention deficit hyperactivity disorder. Child Adolesc Psychiatr Clin N Am 17:261-284.

Polanczyk G, de Lima MS, Horta BL, Biederman J and Rohde LA (2007a) The worldwide prevalence of ADHD: A systematic review and metaregression analysis. Am J Psychiatry 164:942-948.

Polanczyk G, Zeni C, Genro JP, Guimarães AP, Roman T, Hutz MH and Rohde LA (2007b) 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.

Polanczyk G, Laranjeira R, Zalesk IM, Pinsky I, Caetano R and Rohde LA (2010) ADHD in a representative sample of the Brazilian population: Estimated prevalence and comparative adequacy of criteria between adolescents and adults according to the *i.e.* response theory. Int J Meth Psychiatr Res 19:177-184.

Rohde LA, Biederman J, Busnello EA, Zimmermann H, Schmitz M, Martins S and Tramontina S (1999) ADHD in a school sample of Brazilian adolescents: A study of prevalence, comorbid conditions, and impairments. J Am Acad Child Adolesc Psychiatry 38:716-722.

Rohde LA, Roman T, Szobot C, Cunha RD, Hutz MH and Biederman J (2003) Dopamine transporter gene, response to methylphenidate and cerebral blood flow in attention-deficit/hyperactivity disorder: A pilot study. Synapse 48:87-89.

Rohde LA, Szobot C, Polanczyk G, Schmitz M, Martins S and Tramontina S (2005) Attention-deficit/hyperactivity disorder in a diverse culture: Do research and clinical findings support the notion of a cultural construct for the disorder? Biol Psychiatry 57:1436-1441.

Roman T, Schmitz M, Polanczyk G, Eizirik M, Rohde LA and Hutz MH (2001) ADHD: A study of association with both the dopamine transporter gene and the dopamine D4 receptor gene. Am J Med Genet 105:471-478.

Roman T, Schmitz M, Polanczyk GV, Eizirik M, Rohde LA and Hutz MH (2002a) Further evidence for the association between attention-deficit/hyperactivity disorder and the dopamine-beta-hydroxylase gene. Am J Med Genet 114:154-158.

Roman T, Szobot C, Martins S, Biederman J, Rohde LA and Hutz MH (2002b) Dopamine transporter gene and response to methylphenidate in attention-deficit/hyperactivity disorder. Pharmacogenetics 12:497-499.

Roman T, Schmitz M, Polanczyk G, Eizirik M, Rhode LA and Hutz MH (2003) Is the a-2A Adrenergic Receptor Gene (*ADRA2A*) Associated With Attention-Deficit/Hyperactivity Disorder? Am J Med Genet Part B Neuropsychiatr Genet 120B:116-120.

Roman T, Polanczyk G, Zeni C, Genro JP, Rhode LA and Hutz MH (2006) Further evidence of the involvement of alpha-2Aadrenergic receptor gene (*ADRA2A*) in inattentive dimensional scores of attention-deficit/hyperactivity disorder. Mol Psychiatry 11:8-10.

Salatino-Oliveira A, Genro JP, Zeni C, Polanczyk GV, Chazan R, Guimarães AP, Callegari-Jacques SM, Rohde LA and Hutz MH (2011) Catechol-O-Methyltransferase Valine158Methionine polymorphism moderates Methylphenidate effects on pppositional symptoms in boys with Attention-Deficit/Hyperactivity Disorder. Biol Psychiatry 70:216-221.

Schmitz M, Denardin D, Silva TL, Pianca T, Roman T, Hutz MH, Faraone SV and Rohde LA (2006) Association between Alpha-2a-adrenergic Receptor Gene and ADHD Inattentive Type. Biol Psychiatry 60:1028-1033.

Szobot C, Roman T, Cunha R, Acton P, Hutz M and Rohde LA (2005) Brain perfusion and dopaminergic genes in boys with attention-deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 132B:53-58.

Szobot CM, Roman T, Hutz MH, Genro JP, Shih MC, Hoexter MQ, Júnior N, Pechansky F, Bressan RA and Rohde LA (2011) Molecular imaging genetics of methylphenidate response in ADHD and substance use comorbidity. Synapse 65:154-159.

Tovo-Rodrigues L, Rohde LA, Roman T, Schmitz M, Polanczyk G, Zeni C, Marques FZ, Contini V, Grevet EH, Belmonte-de-Abreu P, *et al.* (2011) Is there a role for rare variants in *DRD4* gene in the susceptibility for ADHD? Searching for an effect of allelic heterogeneity. Mol Psychiatr 17:520-526.

Winsberg BG and Comings DE (1999) Association of the Dopamine Transporter Gene (DAT1) with poor methylphenidate response. J Am Acad Child Adolesc Psychiatry 38:1474-1477.

Zeni CP, Guimarães AP, Polanczyk GV, Genro JP, Roman T, Hutz MH and Rohde LA (2007) No significant association between response to methylphenidate and genes of the dopaminergic and serotonergic systems in a sample of Brazilian children with attention-deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 144B:391-394.