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

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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].

Developmentally inappropriate and impairing levels of symptoms in two behavior dimensions - inattention 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 observed. 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 (5HT1B), serotonin transporter (5HTT), synaptosomal-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 Clinicas 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 (MspI, 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-
tons, 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 compared, 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 2 - Pharmacogenetic studies performed in Brazilian children and adolescents.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Approach</th>
<th>Main results</th>
<th>References</th>
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<tbody>
<tr>
<td>ADRA2A</td>
<td>ADHD patients treated with MPH, outcome measures by SNAP IV and SERS, assessed at baseline and 1 and 3 months after the intervention</td>
<td>A significant interaction effect between the presence of MspI G allele and treatment with MPH over time on inattentive scores during the 3 months of treatment</td>
<td>Polanczyk et al., 2007b</td>
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<tr>
<td>ADRA2A</td>
<td>ADHD-I+ patients treated with MPH, outcome measures by inattentive subscale of SNAP IV, assessed at baseline and 1 month after the intervention</td>
<td>Significantly lower inattentive scores with MPH treatment at the first month of treatment in patients with MspI G allele</td>
<td>Da Silva et al., 2008</td>
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<tr>
<td>5HTT, 5HTR2A, 5HTR1B</td>
<td>ADHD patients treated with MPH, outcome measures by SNAP IV, CGAS and SERS, assessed at baseline and 1 month after the intervention</td>
<td>No significant association was detected between polymorphisms assessed (5-HTTLPR, 5HTR1B 861G&gt;C, 5HTR2A His452Tyr) and both response and side effects to MPH</td>
<td>Zeni et al., 2007</td>
</tr>
<tr>
<td>MAOA*</td>
<td>ADHD patients treated with MPH, outcome measures by oppositional subscale of SNAP IV, assessed at baseline and 1 and 3 months after the intervention</td>
<td>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</td>
<td>Guimarães et al., 2009b</td>
</tr>
<tr>
<td>COMT</td>
<td>ADHD patients treated with MPH, outcome measures by oppositional subscale of SNAP IV, assessed at baseline and 1 and 3 months after the intervention</td>
<td>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</td>
<td>Salatino-Oliveira et al., 2011</td>
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Most of the authors attribute the inconsistencies in associations between several genes and ADHD to 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 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.

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

<table>
<thead>
<tr>
<th>Publications worldwide</th>
<th>Year</th>
<th>Publications PRODAH</th>
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<tr>
<td>Cook et al. 1995: First genetics association study with DAT1</td>
<td>1995</td>
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<tr>
<td>La Hoste et al., 1996: association study with DRD4</td>
<td>1996</td>
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<tr>
<td>Brookes et al., 2006: International Multi-centre ADHD Gene project (IMAGE) analyse 51 genes and report association with DAT1, DRD4 and 16 other</td>
<td>2006</td>
<td>Roman et al., 2006: Association between ADRA2A and symptom scores</td>
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<tr>
<td>Faraone et al., 2008: Linkage analysis of ADHD with no significant results</td>
<td>2008</td>
<td>Genro et al., 2007: Association with DAT1 promoter region</td>
</tr>
<tr>
<td>Gizer et al., 2009: meta-analytic review confirming associations with several candidate genes and high heterogeneity between the studies</td>
<td>2009</td>
<td>Guimarães et al., 2009: Association of MAOA with MPH treatment</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>Salatino-Oliveira et al., 2011: Association between COMT and MPH response with oppositional scores</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>Tovo-Rodrigues et al., 2012: association between DRD4 rare variants and ADHD</td>
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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).

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.

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