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**GENE *DRD4* E O USO DE SUBSTÂNCIAS EM ADULTOS COM TDAH**

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## **Artigo Científico**

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### ***DRD4* gene and comorbid substance use in adult ADHD**

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## **Highlights**

- The short allele of 120 bp tandem duplication polymorphism was associated with substance use disorder within an ADHD sample.
- The 120 bp duplication was not associated with ADHD within a sample of crack/cocaine addicts.
- The 120 bp duplication was not associated with susceptibility to ADHD or to crack/cocaine addiction.

## **Abstract**

**Background:** Most individuals with attention-deficit/hyperactivity disorder (ADHD) present one or more psychiatric comorbidities and substance use disorder (SUD) is one of the most frequent in adults. The dopaminergic system is related to the pathogenesis of externalizing disorders, which includes both ADHD and SUD. Thus, the aim of this study was to evaluate the effects of the 120 bp tandem duplication of the *DRD4* gene in the susceptibility to ADHD in adults and to crack/cocaine addiction, as well as to evaluate the associated comorbidities in these two independent samples.

**Methods:** The sample is composed by 555 adults with ADHD, 296 crack/cocaine addicts and 635 blood donors as control group. Association analysis for primary outcomes and comorbidities were performed using binary logistic regression.

**Results:** Regarding susceptibility to ADHD and crack/cocaine addiction, we did not observe a significant association between the 120 bp duplication polymorphism and the disorders ( $p = 0.669$ ,  $OR = 0.95$ ;  $p = 0.559$ ,  $OR = 1.167$ ; respectively). However, evaluating the presence of comorbid SUD within the ADHD sample, it was observed that short allele carriers are at higher risk of developing SUD than homozygotes for the long allele ( $p = 0.023$ ;  $OR = 1.679$ ) and this effect was stronger for non-alcohol substance use ( $p = 0.010$ ;  $OR = 2.085$ ).

**Conclusion:** Our results suggest that the short allele of the 120 bp tandem duplication of *DRD4* gene influences the susceptibility to SUD when in simultaneous presence of ADHD.

**Keywords:** Attention-deficit/hyperactivity disorder, substance use disorder, crack/cocaine addiction, Dopamine D4 Receptor, 120 bp tandem duplication, association analysis.

## 1. Introduction

Genetic factors are involved in the susceptibility and modulation of most psychiatric disorders. In this sense, several genetic association studies have been conducted to better understand this contribution, with the effects of many genetic variants being detected (Price et al., 2015; Sullivan et al., 2014). Nevertheless, the majority of variants associated with psychiatric conditions show small effects and many of these were not further replicated (Manolio et al., 2009; Visscher et al., 2012). The inconsistency between study results may be due to the fact that complex disorders are the outcome of elaborated interplay involving factors such as pleiotropy, epistasis, and gene-environment interactions (Acosta et al., 2004). The effect of this complex interplay, in which pleiotropic or epistatic mechanisms act along with environmental factors, may explain the high prevalence of comorbidities in psychiatric disorders. Moreover, the presence of overlapping genetic factors in complex disorders indicates that the same gene or genes might be associated with diverse and simultaneous phenotypes (Sivakumaran et al., 2011; Stevenson et al., 1993).

Important progress has been made to elucidate the etiological underpinnings of several mental disorders and advances in behavioural genetics are giving support to a trait-based approach regarding the characterization of psychopathologies (Beauchaine and Thayer, 2015). Patterns of functional interaction among a restricted number of brain systems are shown to lead to vulnerability to either or both internalizing and externalizing behaviours (Beauchaine and Thayer, 2015). As an example, impulsivity is a trait that confers vulnerability to externalizing disorders, such as the hyperactive/impulsive and combined presentations of attention-deficit/hyperactivity disorder (ADHD), substance use disorders (SUD), conduct disorder and oppositional defiant disorder (Beauchaine and McNulty, 2013; Zisner and Beauchaine, 2016).

ADHD is a highly prevalent and heritable neuropsychiatric disorder. Most of the individuals with ADHD present one or more psychiatric comorbidities and SUD is one of the most frequent in adults (Kessler et al., 2006). Moreover, adults with ADHD are at higher risk of developing a SUD than individuals without ADHD (Biederman et al., 1998; Charach et al., 2011).

As mentioned above, psychiatric disorders associated with comorbidities are more likely to be influenced by pleiotropic effects of common genes. In this sense, dopamine receptors genes have been extensively studied in psychiatric genetics and can be considered as pleiotropic genes (Abdolmaleky et al., 2005). One of the main pathophysiology theories of ADHD suggests that irregularities of dopamine

neurotransmission are in the centre of the neurobiology of this disorder (Tripp and Wickens, 2009). Furthermore, it is known that the hypofunctioning of the mesolimbic dopamine system is implicated in the pathogenesis of externalizing disorders, including ADHD and SUD (Zisner and Beauchaine, 2016). Following this theory, several candidate gene association studies have reported effects of variants in the dopamine D4 receptor gene (*DRD4*) in ADHD susceptibility (Kirley et al., 2002; Stark et al., 2011), including a meta-analysis (Gizer et al., 2009). Polymorphisms in this gene also have been associated with substance use, involving opioid abuse (Kotler et al., 1997), cigarette smoking (Shields et al., 1998), urge to drink (Hutchison et al., 2002) and the use of other illicit drugs (Mallard et al., 2016). Other associations with behavioural disorders, such as schizophrenia, major depressive disorder (Gatt et al., 2015) and pathological gambling (Perez de Castro et al., 1997) have also been reported.

The majority of studies with ADHD have focused on the variable number of tandem repeats (VNTR) polymorphism of 48 bp located in the third exon of *DRD4*, in which the 7-repeat variant was considered the risk allele in many investigations. This is in line with the evidence showing that this variant encodes a receptor subsensitive to endogenous dopamine (Asghari et al., 1995). Another polymorphism studied in the *DRD4* gene, is a tandem duplication of 120 bp located 1.2 kb upstream of the start codon (Seaman et al., 1999). The duplicated region contains consensus sequences for binding sites of several transcription factors (Barr et al., 2001), thus influencing the transcription rates. The long allele (240 bp) of this polymorphism presents a lower transcriptional activity *in vitro* when compared to the short allele (120 bp) (D'Souza et al., 2004; Kereszturi et al., 2007). Several studies verified the influence of the 120 bp duplication in psychiatric disorders, and it has been associated to ADHD (Ghosh et al., 2013; Kereszturi et al., 2007; Kustanovich et al., 2004; Loo et al., 2008; Mccracken et al., 2000), SUD (Li et al., 2004; Prasad et al., 2013) and schizophrenia (Lai et al., 2010).

Considering the evidence that externalizing disorders can share common genetic factors (Acosta et al., 2004; Arcos-Burgos et al., 2012) and that a dysfunction of dopaminergic system is related to the pathogenesis of these disorders (Zisner and Beauchaine, 2016), we hypothesize that a decrease in the levels of dopaminergic receptors, as a result of altered transcription of *DRD4* gene, could influence the susceptibility to externalizing disorders. In this sense, the aim of this study was to evaluate the effects of the 120 bp tandem duplication of the *DRD4* gene in the susceptibility to ADHD in adults and to crack/cocaine addiction, as well as to evaluate the associated comorbidities in these two independent samples.

## **2. Material and Methods**

### **2.1 Sample**

The sample is composed by 555 adults with ADHD recruited from the Adult Division of the ADHD Outpatient Program at the Hospital de Clínicas de Porto Alegre (HCPA). The included individuals were 18 years or older and are unrelated Brazilians of European descent, diagnosed according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) criteria (APA, 1994). Diagnostic procedures were carried out through semi-structured interviews with the Portuguese version of the Schedule for Affective Disorders and Schizophrenia for School-Age Children – Epidemiological version (K-SADS-E) for ADHD (Grevet et al., 2005). Other psychiatric comorbidities were assessed through SCID-I (Structured Clinical Interview for DSM Axis I Disorders). Individuals were excluded if they presented a neurological disorder with the potential to affect cognition (e.g., delirium, dementia, epilepsy, head trauma, multiple sclerosis), history of psychosis and/or an estimated IQ score lower than 70. Detailed sample characteristics are presented in Table 1.

The control group comprises 635 blood donors of the same hospital where cases were recruited. The inclusion and exclusion criteria were similar to the ADHD group, except for the DSM-IV ADHD diagnosis. Other psychiatric disorders were examined through the structured clinical interview for DSM-IV screening module – SCID-I for the Axis I psychiatric disorders (First et al., 2002) (Table 1).

This study also included a sample composed by 296 crack/cocaine addicts who sought specialized treatment in free and voluntary hospital addiction units. Diagnoses were based on clinical and structured interviews following the DSM-IV criteria. The Adult ADHD Self-Report Scale (ASRS) was used for the screening of ADHD and other psychiatric disorders were evaluated using the Mini-International Neuropsychiatric Interview (MINI). The included subjects in this study had a positive result for cocaine in urine test, were diagnosed with crack/cocaine addiction according to the DSM-IV and are white Brazilians aged 18 years or older. Individuals were excluded if they presented schizophrenia or other psychotic disorders and/or severe cognitive deficit that would impair the capacity of answering the instruments (Table 1).

This work was carried out in accordance with the Declaration of Helsinki and all participants signed an informed consent approved by the Research Ethics Committee of the participating institutions.

## 2.2 Genotyping and SNP selection

The polymorphism evaluated was the 120 bp tandem duplication in *DRD4* gene. It was selected based on the following criteria: previous evidence of association with psychiatric disorders, description of SNP functionality and minor allele frequency higher than 10%. DNA extraction was carried out from peripheral blood using the salting out method (Lahiri and Nurnberger, 1991). The polymorphism was genotyped by Polymerase Chain Reaction (PCR) according to Seaman et al. (1999), followed by 1.5% agarose gel electrophoresis. Genotype distributions were in Hardy-Weinberg Equilibrium.

## 2.3 Statistical analyses

Association analyses between the polymorphism and the outcome (ADHD diagnosis or crack/cocaine dependence) were performed using binary logistic regression in SPSS software version 18.0 (IBM Corp, 2011). Potential confounders were included as covariates when associated ( $p < 0.2$ ) with both the outcome and the evaluated polymorphism (Maldonado and Greenland, 1993). Considering ADHD as the outcome, the potential confounders evaluated were age, gender, ADHD subtype, SUD, and other comorbid conditions (e.g. anxiety and mood disorders). For the crack/cocaine dependence outcome, subjects diagnosed with any SUD in the control sample were excluded from the case-control analysis and the potential confounders evaluated were age, gender, nicotine use and ADHD.

Additionally, the comorbidities evaluated within the ADHD sample were SUDs, which include alcohol dependence/abuse and non-alcohol substance use disorders, with the exception of nicotine dependence that was analysed separately. In this analysis the potential confounders investigated were age, gender and ADHD subtype. In further analysis restricted to the crack/cocaine addicts sample, it was evaluated the presence of ADHD as a comorbidity and the potential confounders assessed were age, gender and nicotine use.

All tests were carried out grouping genotypes according to their frequencies (i.e. heterozygotes and homozygotes for the minor allele were assembled and compared to homozygotes for the major allele).

## 3. Results

We conducted an association analysis between the *DRD4* 120 bp tandem duplication and ADHD in order to assess the effects of this polymorphism on ADHD susceptibility. The case-control analysis did not reveal a significant association



( $p=0.669$ ; OR (CI<sub>95%</sub>)=0.95 (0.74-1.21); Table 2). However, when evaluating comorbidities within the ADHD sample, it was observed that short allele (S) carriers are at higher risk of developing SUD than homozygotes for the long allele (L) ( $p=0.023$ ; OR (CI<sub>95%</sub>)=1.68 (1.07-2.63); Table 3). We analysed the use of different substances separately and observed that the detected effect seems to be related to non-alcohol substance use disorders ( $p=0.010$ ; OR (CI<sub>95%</sub>)=2.08 (1.19-3.64); Table 3), considering that there is no significant difference for alcohol dependence/abuse ( $p=0.150$ ; OR (CI<sub>95%</sub>)=1.44 (0.88-2.38); Table 3) or nicotine dependence ( $p=0.385$ ; OR (CI<sub>95%</sub>)=1.16 (0.82-1.65); Table 3).

Following, in order to further understand the effects of the 120 bp duplication in SUD we evaluated its effects on crack/cocaine addiction. The case-control analysis did not show a significant association between the evaluated polymorphism and SUD susceptibility ( $p=0.559$ ; OR (CI<sub>95%</sub>)=0.92 (0.69-1.22); Table 4). When evaluating the presence of ADHD as comorbidity in the crack/cocaine addicts sample, we did not find a significant association between the 120 bp duplication and ADHD in this sample ( $p=0.111$ ; OR (CI<sub>95%</sub>)=1.601 (0.89-2.85); Table 5).

#### **4. Discussion**

The present study demonstrated that the 120 bp tandem duplication of *DRD4* gene influences the susceptibility to SUD when in concomitant presence of ADHD, in which the short allele carriers seem to have higher risk of developing SUD than homozygotes for the long allele. This effect on SUD susceptibility seems to be related to the presence of both disorders jointly as no association was observed when analyzing SUD in a sample of crack/cocaine addicts regardless of comorbidities. We explored whether the 120 bp tandem duplication would also be associated with comorbid ADHD in the crack/cocaine sample, but no significant results were found.

The lack of association between the 120 bp tandem duplication and susceptibility to ADHD or to crack/cocaine addiction, along with our findings on the comorbid presence of ADHD and SUD, reinforce the need for investigation of predictors of comorbidity. The higher frequency of comorbidities in psychiatric disorders in addition to evidence from Genome-Wide Association Studies (GWAS) demonstrating that a considerable proportion of the genetic variants affecting comorbid phenotypes do not affect the phenotype alone (Edwards et al., 2012) also is consistent with this idea. It is particularly important to investigate these predictors when the psychiatric disorders have such negative consequences like SUDs. Several authors mention the importance of conduct disorder as a predictor of SUDs (August

et al., 2006; Costello et al., 2003; Looby, 2008). In this sense, Brook et al. (2010) observed that conduct disorder plays a mediating role in the association between ADHD and SUDs. Ghosh et al. (2013) found an association of the short allele of the 120 bp duplication with conduct disorder in childhood ADHD. Interestingly, the short allele was associated to SUD comorbidity in ADHD in our study. These results could represent shared underlying mechanisms between comorbid conduct disorder and SUD in ADHD.

To our knowledge, this is the first study exploring the effects of the 120 bp duplication on comorbid SUD in adults with ADHD. Several studies have explored the influence of this polymorphism in ADHD alone, but the results are controversial. Similar to the results presented here, some studies found no association (Barr et al., 2001; Bhaduri et al., 2006; Brookes et al., 2005; Todd et al., 2001) while others identified the long or the short allele as a risk factor for ADHD (Kereszturi et al., 2007; Kustanovich et al., 2004; Mccracken et al., 2000). The meta-analysis performed by Gizer et al. (2009) did not show significant association between childhood ADHD and this polymorphism. However, a significant effect of heterogeneity was observed, indicating that differences between studies may explain discrepancies in the results. Thus, the lack of association and the inconsistency between reports could be due to clinical differences of the sample, for example, regarding the comorbidity profile. Another meta-analysis with ADHD, this time in adults, also did not implicate the polymorphism with the disorder (Sanchez-Mora et al., 2011).

Considering other psychiatric disorders, especially studies related to SUD, no association was found with heroin dependence (Szilagyí et al., 2005; Vereczkei et al., 2013), whereas other studies suggest that the long allele may confer susceptibility to alcohol abuse/dependence (Prasad et al., 2013) and the short allele may be related to higher scores of novelty seeking in alcoholic families (Rogers et al., 2004), as well as to methamphetamine abuse in a haplotypic analysis (Li et al., 2004). The overall scenario regarding association studies of the 120 bp duplication with psychiatric disorders does not allow a consensus on the associated risk allele. This might be explained by the clinical heterogeneity, or other unexplained genetic factors, such as pleiotropy, epistasis, and gene-environment interactions.

Although our results are related to the short allele of the 120 bp duplication, the long allele of this polymorphism has been reported to decrease the transcriptional activity of the *DRD4* gene (D'Souza et al., 2004; Kereszturi et al., 2007) and a hypofunctioning mesolimbic dopamine system has been implicated in the pathogenesis of externalizing disorders (Zisner and Beauchaine, 2016). However,

considering that psychiatric disorders are complex phenotypes, other factors are certainly involved with the susceptibility and modulation of these disorders (Acosta et al., 2004), as mentioned above. Additionally, only one polymorphism was analysed in the present study, which did not account for the possible effects of gene-gene interactions.

One limitation of this study that should be considered is the small sample size of the subgroups analysed within the ADHD and the crack/cocaine addicts samples. These modest sizes limited the statistical power of the results and it may be related to the lack of association of the 120 bp duplication with comorbid ADHD in the crack/cocaine sample. Thus, further investigations with larger samples should evaluate the role of this polymorphism in comorbid phenotypes.

## **5. Conclusions**

Our results suggest that the short allele of the 120 bp tandem duplication of *DRD4* gene influences the susceptibility to SUD when in simultaneous presence of ADHD. Moreover, this result could represent shared underlying mechanisms between comorbid conduct disorder and SUD in ADHD. Thus, the current findings should be considered in future genetics investigations regarding comorbidities in ADHD and predictors for other psychiatric disorders.

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## **Contributors**

CW, DBK, BSS and CHDB designed the study; BSV, LD, FHPK, ESV, RGO, LAR and EHG coordinated the collection of samples; CW and APA extracted DNA from blood; CW genotyped the sample; CW, DBK and BSS conducted statistical analyses and drafted the first version of the manuscript. All contributors have made a substantial intellectual contribution to the work. All authors have participated in manuscript writing by reviewing drafts and approving this final version.

## **Conflict of interest**

The author(s) declare the following potential conflict of interest with respect to the research, authorship and/or publication of this article: Dr. Grevet was on the speaker's bureau for Novartis and Shire for the last 3 years. He also received travel awards (air tickets and hotel accommodations) for participating in two psychiatric meetings from Shire and Novartis. Dr. Rohde has received Honoraria, has been on the speakers' bureau/advisory board and/or has acted as a consultant for Eli-Lilly, Janssen-Cilag, Novartis and Shire in the last three years. He receives authorship royalties from Oxford Press and ArtMed. He also received travel awards for taking part of 2014 APA and 2015 WFADHD meetings from Shire. The ADHD and Juvenile Bipolar Disorder Outpatient Programs chaired by him received unrestricted educational and research support from the following pharmaceutical companies in the last three years: Eli-Lilly, Janssen-Cilag, Novartis, and Shire. All other authors report no biomedical financial interests or potential conflicts of interest.

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## References

- Abdolmaleky, H., Thiagalingam, S., Wilcox, M., 2005. Genetics and Epigenetics in Major Psychiatric Disorders. *AM J Pharmacogenomics* 5, 149–160.
- Acosta, M.T., Arcos-burgos, M., Muenke, M., 2004. Attention deficit/hyperactivity disorder (ADHD): Complex phenotype, simple genotype? *Genet. Med.* 6, 1–15. doi:10.1097/01.GIM.0000110413.07490.0B
- APA, 1994. *Diagnostic and Statistical Manual of Mental Disorders*, 4<sup>a</sup> ed., American Psychiatric Association, Washington, DC.
- Arcos-Burgos, M., Velez, J., Solomon, B.D., Muenke, M., 2012. A common genetic network underlies substance use disorders and disruptive or externalizing disorders. *Hum. Genet.* 131, 917–929. doi:10.1007/s00439-012-1164-4
- Asghari, V., Sanyal, S., Buchwaldt, S., Paterson, A., Jovanovic, V., Tol, H.H.M. Van, 1995. Modulation of Intracellular Cyclic AMP Levels by Different Human Dopamine D4 Receptor Variants. *J. Neurochem.* 65, 1157–1165.
- August, G.J., Winters, K.C., Realmuto, G.M., Fahnhorst, T., A, A.B.M., Lee, S., 2006. Prospective Study of Adolescent Drug Use Among Community Samples of ADHD and Non-ADHD Participants. *J. Am. Acad. Child Adolesc. Psychiatry* 45, 824–832. doi:10.1097/01.chi.0000219831.16226.f8
- Barr, C.L., Feng, Y., Wigg, K.G., Schachar, R., Tannock, R., Roberts, W., Malone, M., Kennedy, J.L., 2001. 5'-Untranslated Region of the Dopamine D4 Receptor Gene and Attention-Deficit Hyperactivity Disorder. *Am. J. Med. Genet. (Neuropsychiatric Genet.)* 105, 84–90.
- Beauchaine, T.P., McNulty, T., 2013. Comorbidities and continuities as ontogenic processes: Toward a developmental spectrum model of externalizing psychopathology. *Dev. Psychopathol.* 25, 1505–1528. doi:10.1017/S0954579413000746.Comorbidities
- Beauchaine, T.P., Thayer, J.F., 2015. Heart rate variability as a transdiagnostic biomarker of psychopathology. *Int. J. Psychophysiol.* doi:10.1016/j.ijpsycho.2015.08.004
- Bhaduri, N., Das, M., Sinha, S., Chattopadhyay, A., Gangopadhyay, P.K., Chaudhuri, K., Singh, M., Mukhopadhyay, K., 2006. Association of Dopamine D4 Receptor (DRD4) Polymorphisms With Attention Deficit Hyperactivity Disorder in Indian Population. *Am. J. Med. Genet. (Neuropsychiatric Genet.)* 61–66. doi:10.1002/ajmg.b.30225
- Biederman, J., Wilens, T.E., Mick, E., Faraone, S. V, Spencer, T., 1998. Does Attention-Deficit Hyperactivity Disorder Impact the Developmental Course of Drug and Alcohol Abuse and Dependence? *Am. J. Psychiatry* 155, 269–273.

- Brook, D., Brook, J.S., Zhang, C., Koppel, J., 2010. The association between Attention Deficit Hyperactivity Disorder in Adolescence and Substance Use Disorders in Adulthood. *Arch Pediatr Adolesc Med.* 164, 930–934. doi:10.1001/archpediatrics.2010.180.
- Brookes, K., Xu, X., Chen, C., Huang, Y., Wu, Y., Asherson, P., 2005. No evidence for the association of DRD4 with ADHD in a Taiwanese population within-family study. *BMC Med. Genet.* 6, 1–6. doi:10.1186/1471-2350-6-31
- Charach, A., Yeung, E., Climans, T., Lillie, E., 2011. Childhood Attention-Deficit/Hyperactivity Disorder and Future Substance Use Disorders: Comparative Meta-Analyses. *J. Am. Acad. Child Adolesc. Psychiatry* 50, 9–21. doi:10.1016/j.jaac.2010.09.019
- Costello, E.J., Mustillo, S., Erkanli, A., Keeler, G., Angold, A., 2003. Prevalence and Development of Psychiatric Disorders in Childhood and Adolescence. *Arch Gen Psychiatry* 60, 837–844.
- D'Souza, U.M., Russ, C., Tahir, E., Mill, J., McGuffin, P., Asherson, P.J., Craig, I.W., 2004. Functional effects of a tandem duplication polymorphism in the 5'flanking region of the DRD4 gene. *Biol. Psychiatry* 56, 691–7. doi:10.1016/j.biopsych.2004.08.008
- Edwards, S., Guerrero, M., Ghoneim, O.M., Roberts, E., Koob, G.F., 2012. Evidence that Vasopressin V1b Receptors Mediate the Transition to Excessive Drinking in Ethanol-Dependent Rats. *Addict. Biol.* 17, 76–85. doi:10.1111/j.1369-1600.2010.00291.x.
- First, M.B., Spitzer, R.L., Gibbon Miriam, and Williams. Janet BW. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition with Psychotic Screen (SCID-I/PW/PSY Screen) New York: Biometrics Research, New York State Psychiatric Institute
- Gatt, J.M., Burton, K.L.O., Williams, L.M., Scho, P.R., 2015. Specific and common genes implicated across major mental disorders: A review of meta-analysis studies 60, 1–13. doi:10.1016/j.jpsychires.2014.09.014
- Ghosh, P., Sarkar, K., Bhaduri, N., Ray, A., Sarkar, K., Sinha, S., Mukhopadhyay, K., 2013. Catecholaminergic Gene Variants : Contribution in ADHD and Associated Comorbid Attributes in the Eastern Indian Probands 2013.
- Gizer, I.R., Ficks, C., Waldman, I.D., 2009. Candidate gene studies of ADHD: a meta-analytic review. *Hum. Genet.* 126, 51–90. doi:10.1007/s00439-009-0694-x
- Grevet, E.H., Bau, C.H.D., Salgado, C.A.I., Ficher, A., Victor, M.M., Garcia, C., Sousa, N.O. de, Nerung, L., Belmonte-de-Abreu, P., 2005. O Diagnóstico em Adultos do Transtorno de Déficit de Atenção/Hiperatividade e Transtorno de

- Oposição desafiante utilizando o K-SADS-E. *Arq Neuropsiquiatr* 63, 307–310.
- Hutchison, K.E., Mcgeary, J., Smolen, A., Bryan, A., Swift, R.M., 2002. The DRD4 VNTR Polymorphism Moderates Craving After Alcohol Consumption. *Heal. Psychol.* 21, 139–146. doi:10.1037//0278-6133.21.2.139
- IBM Corp, 2011. IBM SPSS Statistics for Windows.
- Kereszturi, E., Kiraly, O., Csapo, Z., Tarnok, Z., Gadoros, J., Sasvari-szekely, M., Nemoda, Z., 2007. Association Between the 120-bp Duplication of the Dopamine D4 Receptor Gene and Attention Deficit Hyperactivity Disorder: Genetic and Molecular Analyses. *Am. J. Med. Genet. (Neuropsychiatric Genet.* 231–236. doi:10.1002/ajmg.b.30444
- Kessler, R.C., Adler, L., Barkley, R., Biederman, J., Conners, C.K., Demler, O., Faraone, S. V, Greenhill, L.L., Howes, M.J., Secnik, K., Spencer, T., Ustun, T.B., Walters, E.E., Zaslavsky, A.M., 2006. The Prevalence and Correlates of Adult ADHD in the United States: Results From the National Comorbidity Survey Replication. *Am J Psychiatry* 163, 716–723. doi:10.1176/appi.ajp.163.4.716.
- Kirley, A., Hawi, Z., Daly, G., McCarron, M., Mullins, C., Millar, N., Waldman, I., Fitzgerald, M., Gill, M., 2002. Dopaminergic System Genes in ADHD: Toward a Biological Hypothesis. *Neuropsychopharmacology* 27, 607–619.
- Kotler, M., Cohen, H., Segman, R., Gritsenko, I., Hospital, E., 1997. Excess dopamine D4 receptor (D4DR) exon III seven repeat allele in opioid-dependent subjects. *Mol. Psychiatry* 251–254.
- Kustanovich, V., Ishii, J., Crawford, L., Yang, M., MCGough, J.J., Mccracken, J.T., Smalley, S.L., 2004. Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD : confirmation of association of ADHD with DRD4 and DRD5. *Mol. Psychiatry* 711–717. doi:10.1038/sj.mp.4001466
- Lahiri, D.K., Nurnberger, J.I., 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* 19, 5444. doi:10.1093/nar/19.19.5444
- Lai, J.H., Zhu, Y.S., Huo, Z.H., Sun, R.F., Yu, B., Wang, Y.P., Chai, Z.Q., Li, S.B., 2010. Association study of polymorphisms in the promoter region of DRD4 with schizophrenia , depression , and heroin addiction. *Brain Res.* 227–232. doi:10.1016/j.brainres.2010.08.064
- Li, T., Chen, C., Hu, X., Ball, D., Lin, S., Chen, W., Sham, P.C., Loh, E., Murray, R.M., Collier, D.A., 2004. Association Analysis of the DRD4 and COMT Genes in Methamphetamine Abuse 124, 120–124. doi:10.1002/ajmg.b.30024
- Loo, S.K., Rich, E.C., Ishii, J., MCGough, J., Mccracken, J., Nelson, S., Smalley, S.L., 2008. Cognitive functioning in affected sibling pairs with ADHD : familial

- clustering and dopamine genes 9, 950–957. doi:10.1111/j.1469-7610.2008.01928.x
- Looby, A., 2008. Childhood attention deficit hyperactivity disorder and the development of substance use disorders: Valid concern or exaggeration? *Addict. Behav.* 33, 451–463. doi:10.1016/j.addbeh.2007.10.006
- Maldonado, G., Greenland, S., 1993. Simulation study of confounder-selection strategies. *Am J Epidemiol.* 138, 923–36.
- Mallard, T.T., Doorley, J., Esposito-smythers, C.L., Mcgeary, J.E., 2016. Dopamine D4 Receptor VNTR Polymorphism Associated With Greater Risk for Substance Abuse Among Adolescents With Disruptive Behavior Disorders: Preliminary Results. *Am. J. Addict.* 25, 56–61. doi:10.1111/ajad.12320
- Manolio, T. a, Collins, F.S., Cox, N.J., Goldstein, D.B., Hindorff, L. a, Hunter, D.J., McCarthy, M.I., Ramos, E.M., Cardon, L.R., Chakravarti, A., Cho, J.H., Guttmacher, A.E., Kong, A., Kruglyak, L., Mardis, E., Rotimi, C.N., Slatkin, M., Valle, D., Whittemore, A.S., Boehnke, M., Clark, A.G., Eichler, E.E., Gibson, G., Haines, J.L., Mackay, T.F.C., McCarroll, S. a, Visscher, P.M., 2009. Finding the missing heritability of complex diseases. *Nature* 461, 747–53. doi:10.1038/nature08494
- Mccracken, J.T., Smalley, S.L., MCGough, J.J., Crawford, L., Homme, M. Del, Cantor, R.M., 2000. Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene ( DRD4 ) with attention deficit hyperactivity disorder. *Mol. Psychiatry* 531–536.
- Perez de Castro, I., Ibanez, A., Saiz-Ruiz, J., Fernandez-Piqueras, J., 1997. Genetic association study between pathological gambling and a functional DNA polymorphism at the D4 receptor gene. *Pharmacogenetics* 7, 345–348.
- Prasad, P., Ambekar, A., Vaswani, M., 2013. Case – control association analysis of Dopamine receptor polymorphisms in alcohol dependence: a pilot study in Indian males. *BMC Res. Notes* 1–6.
- Price, A.L., Spencer, C.C.A., Donnelly, P., 2015. Progress and promise in understanding the genetic basis of common diseases. *Proc. R. Soc. B* 282, 1–10.
- Rogers, G., Joyce, P., Mulder, R., Sellman, D., Miller, A., Allington, M., Olds, R., Wells, E., Kennedy, M., 2004. Association of a duplicated repeat polymorphism in the 5'-untranslated region of the DRD4 gene with novelty seeking. *Am. J. Med. Genet. (Neuropsychiatric Genet.)* 126B, 95–98. doi:10.1002/ajmg.b.20133
- Sanchez-Mora, C., Ribases, M., Casas, M., Bosch, R., Brunso, L., Jacobsen, K.K., Landaas, E.T., Lundervold, A.J., Gross-lesch, S., Kreiker, S., Jacob, C.P.,



- Lesch, K., Buitelaar, J.K., Hoogman, M., Kiemeneij, L.A.L.M., Kooij, J.J.S., Mick, E., Asherson, P., Faraone, S. V., Franke, B., Reif, A., Johansson, S., Haavik, J., Ramos-quiroya, J.A., Cormand, B., 2011. Exploring DRD4 and Its Interaction With SLC6A3 as Possible Risk Factors for Adult ADHD: A Meta-Analysis in Four European Populations 600–612. doi:10.1002/ajmg.b.31202
- Seaman, M.I., Fisher, J.B., Chang, F., Kidd, K.K., 1999. Tandem Duplication Polymorphism Upstream of the Dopamine D4 Receptor Gene ( DRD4 ). *Am. J. Med. Genet. (Neuropsychiatric Genet.* 88, 705–709.
- Shields, P.G., Lerman, C., Audrain, J., Bowman, E.D., Main, D., Boyd, N.R., Caporaso, N.E., 1998. Dopamine D4 Receptors and the Risk of Cigarette Smoking in African-Americans and Caucasians. *Cancer Epidemiol. Biomarkers Prev.* 7, 453–458.
- Sivakumaran, S., Agakov, F., Theodoratou, E., Prendergast, J.G., Zgaga, L., Manolio, T., Rudan, I., Mckeigue, P., Wilson, J.F., Campbell, H., 2011. Abundant Pleiotropy in Human Complex Diseases and Traits. *Am. J. Hum. Genet.* 89, 607–618. doi:10.1016/j.ajhg.2011.10.004.
- Stark, R., Bauer, E., Merz, C.J., Zimmermann, M., Reuter, M., Plichta, M.M., Kirsch, P., Lesch, K.P., Fallgatter, A.J., Vaitl, D., Herrmann, M.J., 2011. Neuropsychologia ADHD related behaviors are associated with brain activation in the reward system. *Neuropsychologia* 49, 426–434. doi:10.1016/j.neuropsychologia.2010.12.012
- Stevenson, J., Pennington, B.F., Gilger, J.W., DeFries, J.C., Gillis, J.J., 1993. Hyperactivity and Spelling Disability: Testing for Shared Genetic Aetiology. *J. Child Psychol. Psychiatry* 34, 1137–1152.
- Sullivan, P.F., Daly, M.J., O'Donovan, M.C., 2014. Genetic Architectures of Psychiatric Disorders: The Emerging Picture and Its Implications. *Nat. Rev. Genet.* 13, 537–551. doi:10.1038/nrg3240.Genetic
- Szilagyi, A., Boor, K., Szekely, A., Gaszner, P., Kalasz, H., Sasvari-Szekely, M., Barta, C., 2005. Combined Effect of Promoter Polymorphisms in the Dopamine D4 Receptor and the Serotonin Transporter Genes in Heroin Dependence. *Neuropsychopharmacol. Hungarica* 28–33.
- Todd, R.D., Neuman, R.J., Lobos, E.A., Jong, Y.I., Reich, W., Heath, A.C., 2001. Lack of Association of Dopamine D4 Receptor Gene Polymorphisms With ADHD Subtypes in a Population Sample of Twins. *Am. J. Med. Genet. (Neuropsychiatric Genet.* 105, 432–438.
- Tripp, G., Wickens, J.R., 2009. Neurobiology of ADHD. *Neuropharmacology* 57, 579–589. doi:10.1016/j.neuropharm.2009.07.026

- Vereczkei, A., Demetrovics, Z., Szekely, A., Sarkozy, P., Antal, P., Szilagyi, A., Sasvari-szekely, M., Barta, C., 2013. Multivariate Analysis of Dopaminergic Gene Variants as Risk Factors of Heroin Dependence 8. doi:10.1371/journal.pone.0066592
- Visscher, P.M., Brown, M.A., McCarthy, M.I., Yang, J., 2012. Five Years of GWAS Discovery. *Am. J. Hum. Genet.* 90, 7–24. doi:10.1016/j.ajhg.2011.11.029
- Zisner, A., Beauchaine, T.P., 2016. Neural substrates of trait impulsivity, anhedonia, and irritability: Mechanisms of heterotypic comorbidity between externalizing disorders and unipolar depression. *Dev. Psychopathol.* 28, 1177–1208. doi:10.1017/S0954579416000754

**Table 1** - Participants demographic and psychiatric characteristics

	<b>ADHD (N=555)</b>	<b>Crack/Cocaine (N=296)</b>	<b>Controls (N=635)</b>
	<b>Mean (SD)</b>	<b>Mean (SD)</b>	<b>Mean (SD)</b>
<b>Age (years)</b>	33.745 (10.81)	30.740 (7.96)	29.082 (8.66)
	<b>N (%)</b>	<b>N (%)</b>	<b>N (%)</b>
<b>Gender (female)</b>	253 (45.6)	131 (44.3)	325 (51.2)
<b>Substance Use Disorder (SUD)<sup>a</sup></b>	103 (18.6)	–	21 (3.3)
<b>Abuse/Dependence of non-alcohol substances</b>	58 (10.5)	–	8 (1.3)
<b>Abuse/Dependence of alcohol</b>	76 (13.7)	–	15 (2.4)
<b>Use of Nicotine</b>	240 (43.2)	239 (82.7)	112 (17.7)
<b>ADHD</b>	–	90 (43.7)	–

ADHD: Attention Deficit/Hyperactivity Disorder; SD: standard deviation

<sup>a</sup>Except the use of nicotine

**Table 2** - Logistic regression for *DRD4* 120bp tandem duplication effects regarding ADHD susceptibility

	<b>LL N (%)</b>	<b>SS + SL N (%)</b>	<b>OR (CI<sub>95%</sub>)</b>	<b>P-value</b>
<b>Cases</b>	345 (62.2%)	210 (37,8%)	0.95 (0.74-1.21)	0.669
<b>Controls</b>	395 (62.2%)	240 (37.8%)		

L: Long allele (240bp)

S: Short allele (120bp)

OR: Odds Ratio

CI: Confidence Interval

Gender and substance use disorder were included as covariates

**Table 3** – Association of *DRD4* 120bp tandem duplication with substance use comorbidities in individuals with ADHD (N=555)

Comorbidity		N (%)	LL N (%)	SS + SL N (%)	OR (CI <sub>95%</sub> )	P- value
<b>Substance use disorder (SUD)<sup>a</sup></b>	Yes	103 (18.6)	56 (54.4)	47 (45.6)	1.68 (1.07-2.63)	<b>0.023</b>
	No	452 (81.4)	289 (64.1)	162 (35.9)		
<b>Abuse/Dependence of non-alcohol substances</b>	Yes	58 (10.5)	28 (48.3)	30 (51.7)	2.08 (1.19-3.64)	<b>0.010</b>
	No	497 (89.5)	317 (63.8)	180 (36.2)		
<b>Abuse/Dependence of alcohol</b>	Yes	76 (13.7)	43 (56.6)	33 (43.4)	1.44 (0.88-2.38)	0.150
	No	479 (86.3)	302 (63.2)	176 (36.8)		
<b>Use of Nicotine</b>	Yes	240 (43.2)	145 (60.4)	95 (39.6)	1.16 (0.82-1.65)	0.385
	No	315 (56.8)	200 (63.5)	115 (36.5)		

<sup>a</sup>Except the use of nicotine

L: Long allele (240bp)

S: Short allele (120bp)

Gender was included as covariate

**Table 4** - Logistic regression for *DRD4* 120bp tandem duplication effects regarding crack/cocaine addiction susceptibility

	<b>LL N (%)</b>	<b>SS + SL N (%)</b>	<b>OR (CI<sub>95%</sub>)</b>	<b>P-value</b>
<b>Cases</b>	180 (60.8%)	116 (39.2%)	0.92 (0.69-1.22)	0.559
<b>Controls</b>	392 (62.8%)	232 (37.2%)		

L: Long allele (240bp)

S: Short allele (120bp)

OR: Odds Ratio

CI: Confidence Interval

Gender and age were included as covariates

**Table 5** – Association of *DRD4* 120bp tandem duplication with comorbid ADHD in individuals with crack/cocaine addiction (N=206)

Comorbidity		N (%)	LL N (%)	SS + SL N (%)	OR (CI <sub>95%</sub> )	P- value
<b>ADHD</b>	Yes	90 (43.7)	61 (67.8)	29 (32.2)	1.6 (0.89-2.85)	0.111
	No	116 (56.3)	66 (56.9)	50 (43.1)		

L: Long allele (240bp)

S: Short allele (120bp)

OR: Odds Ratio

CI: Confidence Interval

Gender and age were included as covariates