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Insomnia Polygenic Component on Attention Deficit-Hyperactivity Disorder: Exploring this Association Using Genomic Data from Brazilian Families

Marina Xavier Carpena[1,2] Brenda Barbon Fraga[1] Thais Martins-Silva[1,3] Angélica Salatino-Oliveira[4] Júlia Pasqualini Genro[5] Guilherme V. Polanczyk[6] Cristian Zeni[7] Marcelo Schmitz[8] Rodrigo Chazan[9] Mara Helena Hutz[4] Luis Augusto Rohde[10,8] Luciana Tovo-Rodrigues[1]

[1] Postgraduate Program in Epidemiology, Federal University of Pelotas, Pelotas, RS, Brazil
[2] Postgraduate Program in Developmental Disorders, Universidade Presbiteriana Mackenzie, São Paulo, SP, Brazil
[3] Human Development and Violence Research Centre (DOVE), Federal University of Pelotas, Pelotas, RS, Brazil
[4] Department of Genetics, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[5] Postgraduate Program in Biosciences, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil
[6] Department of Psychiatry, Faculdade de Medicina (FMUSP), Universidade de São Paulo, São Paulo, SP, Brazil
[7] Health Science Center Houston, University of Texas, Houston, Texas, United States
[8] ADHD Outpatient Program, Hospital de Clínicas de Porto Alegre, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[9] Postgraduate Program in Psychiatry and Behavioral Sciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[10] National Institute of Developmental Psychiatry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

Address for correspondence Marina Xavier Carpena, PhD (email: marinacarpena_@hotmail.com).

Sleep Sci

Abstract

Introduction Insomnia is highly prevalent among individuals with Attention-Deficit/Hyperactivity Disorder (ADHD). However, the biological mechanisms shared between both conditions is still elusive. We aimed to investigate whether insomnia's genomic component is able to predict ADHD in childhood and adolescence.

Methods A Brazilian sample of 259 ADHD probands and their biological parents were included in the study. Their genomic DNA genotypes were used to construct the polygenic risk score for insomnia (Insomnia PRS), using the largest GWAS summary statistics as a discovery sample. The association was tested using logistic regression, under a case-pseudocontrol design.

Results Insomnia PRS was nominally associated with ADHD (OR = 1.228, $p = 0.022$), showing that the alleles that increase the risk for insomnia also increase the risk for ADHD.

Discussion Our results suggest that genetic factors associated with insomnia may play a role in the ADHD genetic etiology, with both phenotypes likely to have a shared genetic mechanism.

Keywords
- polygenic risk score
- insomnia
- attention-deficit/hyperactivity disorder

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Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Introduction

Insomnia is a sleep disorder characterized by problems in starting or maintaining sleep (with daytime consequences) when there are adequate opportunities and circumstances for sleep.[1] Approximately one-third of the world's population has some symptom of insomnia.[2] This is a multifactorial disorder, as its etiology can include genetic predisposition and environmental influences.[1,2] Recent genome-wide association studies (GWAS) revealed that the associated loci with self-reported insomnia may play a role in the neurons' axonal part, and specific cortical and subcortical tissues.[3] Furthermore, the involvement of brain regions has been confirmed, including the cerebellum and frontal cortex.[4]

Insomnia is highly prevalent in individuals with attention-deficit/hyperactivity disorder (ADHD), which is one of the most common neurodevelopmental disorders in children and adolescents.[5] The prevalence of insomnia reaches 80% in ADHD clinical samples (ranging from 43 to 80%).[6] In addition to clinical evidence showing the high rates of comorbidity between insomnia and ADHD, genetic evidence further strengthened the relationship between both disorders.[3,4,7] Insomnia appears to be the sleep phenotype with the greatest genetic overlap with ADHD, with both having the largest number of genes shared.[7]

Despite the recent increase in the knowledge of ADHD-insomnia genomic relationship,[3,4] there is still little evidence about the effect of genetic susceptibility of insomnia in individuals with ADHD. Most of the studies have evaluated few or a single genetic marker previously associated with the circadian rhythm on ADHD etiology.[8–11] Considering genomic data, genomic liability to other sleep phenotypes have been associated with ADHD.[12,13] However, a recent study showed no evidence of association between polygenic liability for insomnia and ADHD,[12] indicating the current need for further studies with a genomic approach.

The present study aims to investigate the association between genetic markers of insomnia revealed by a recent GWAS,[4] as well as ADHD in a clinical Brazilian sample of children with ADHD and their biological parents. The main hypothesis is that the cumulative effect of markers associated with insomnia is also associated with ADHD.

Material and Methods

Subjects and Study Design

Genomic data from 259 probands with ADHD and their biological parents were included in this study. The probands were recruited from the ADHD Outpatient Program (ProDAH) of the Hospital de Clínicas de Porto Alegre (HCPA), Brazil. The diagnosis was given according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. The assessment process followed a previously reported three-stage protocol, including the application of semi-structured diagnostic interviews (Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime Version, KSADS-PL) by trained research assistants, and clinical assessments by experienced child psychiatrists. The Swanson, Nolan, and Pelham scale version IV (SNAP-IV) was rated by child psychiatrists blinded to genotype to assess symptom severity. This scale has been translated to Portuguese and is considered reliable in a Brazilian sample.[14]

For the current study, the family-based design was converted into case-pseudocontrol, which is an alternative procedure to the genotype transmission/disequilibrium test. In this case, the pseudocontrol is created with the nontransmitted alleles from parents to children.[15]

DNA, Genotyping, and Quality Control

Peripheral blood samples were collected from probands and their parents. Genomic DNA was extracted from lymphocytes by salting out protocol. Infinium PsychArray-24 BeadChip, an array developed for studies focused on psychiatric predisposition and risk, was used to genotype both children and parents, in approximately 593,260 markers. Quality control and imputation were done using the Rapid Imputation Consortium Pipeline (Ricopili), a pipeline developed and used by the Psychiatric Genome Consortium (PGC), which covers both individual and markers quality control (QC). Additional markers were imputed using 1000 Genomes Project European population phase 3 as a reference panel (https://www.internationalgenome.org/category/phase-3/), obtaining a total of 11,799,192 variants. Data were filtered to include only those with **(i)** $< 2\%$ of missing genotypes; **(ii)** minor allele frequency (MAF) ≥ 0.01; and (iii) a Hardy-Weinberg Equilibrium deviation with p-value 1×10^{-6}, using the Plink software, v.1.9, totaling 7,003,490 variants.

Genetic Markers Associated with Insomnia

The Insomnia PRS were created based on the association estimates of genetic markers reported for insomnia in the GWAS study performed by Lane et al. (2019). This study was conducted with participants of European ancestry from the UKBiobank (UKBiobank, Stockport, UK) cohort. In summary, UKBiobank is a prospective study with over 500,000 participants living in the United Kingdom. All individuals registered with the National Health Service who were aged 40 to 69 years and lived within 40km of a study center were invited to participate. More details could be found in Sudlow et al. (2015) and the original paper.[4]

Insomnia was assessed by the self-report of frequent insomnia symptoms, which would better capture the phenotype information, since it includes only people who frequently have insomnia.[4] The frequent symptoms were assessed by the following questions: "*Do you have trouble falling asleep at night?*" or "*Do you wake up in the middle of the night?*," with the following options for answering: "never/rarely," "sometimes," "usually," or "prefer not to answer." Subjects who responded "prefer not to answer" ($n = 637$) were marked as "missing data". As such, the participants were dichotomized into controls ("never/rarely"; $n = 108{,}357$) and frequent insomnia symptoms ("usually"; $n = 129{,}270$). Including a total of 237,627 individuals of European descent, Lane et al. (2019) found 48 genetic loci associated with frequent insomnia symptoms.

Polygenic Risk Score Analysis

To consider the cumulative effect of the variants associated with insomnia, we performed a Polygenic Risk Score (PRS) analysis, using the PRSice software, v. 2.2.1. We included a range of PRSs at increasingly liberal significant *p*-value thresholds (P_T) , which were chosen due to being classical thresholds for statistical analyses in biological or genomic analyses: 5e-8, 5e-6, and 0.05. After the polygenic scores were obtained from the individuals, data distribution was evaluated by the histogram and normalized to z-score. The association was tested using logistic regression using the Stata (StataCorp LLC., College Station, TX, US) software, v.14.0. Effect estimates were given by odds ratio (OR), 95% confidence interval (95% CI), and *p*-value. Pseudo R-squared (Pseudo-R2) statistic was used to indicate the variance explained by PRS of each tested *p*-threshold for logistic regression. The Bonferroni test was used to correct for multiple tests (corresponding to the number of P_T tested: 3) and a p-value < 0.017 was considered statistically significant. Due to the case-pseudocontrol design of our study, it was not necessary to adjust for principal components, due to the cases and pseudocontrols being paired by ancestry, as they belong to the same family.

Research Ethics

All subjects gave their informed consent for inclusion before participating in the study. Parents provided written informed consent and probands provided verbal assent to participate. The study project was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre (HCPA) (Project identification code 45210715.1.0000.5327).

Results

The participating children and adolescents were 10.42-years-old on average (ranging from 4 to 17-years-old). Boys represented 76.4% of the sample. The majority of the participants (83.4%) identified as white, while the remaining probands (16.6%) identified as black or mixed. The ADHD combined and inattentive subtypes were the most frequently observed, present in 47.1 and 44.0% of the sample, respectively. The more frequent comorbidities related to mental health were oppositional defiant disorders, present in 35.5% of the probands, followed by anxiety disorders (27.8%), conduct disorders (13.9%), and mood disorders (8.9%).

►**Table 1** shows the results of the association between insomnia PRSs and ADHD. The increase of risk alleles in the of Insomnia-$PRS_{PT = 0.05}$ was associated with higher odds of this disorder (OR= 1.228, 95%CI = 1.030–1.462; $p = 0.022$). This score explained 0.7% of the ADHD variance in our sample (pseudo-$R^2 = 0.007$). The remaining PRS, based on stricter *p*-value thresholds, also showed a positive association with this disorder, even though they explained lower variance and were not statistically significant (►**Table 1**). No association held up after correction for multiple tests.

Discussion

In this study, we investigated the association between insomnia and ADHD using a genomic approach to uncover the polygenic relationship between both traits. Nominally associated PRS points toward a suggestive genetic overlap between insomnia and this disorder. Even though our results did not hold up after multiple test corrections, it consists of an independent clinical sample that replicates previous findings, confirming an important role of the insomnia genetic component on the etiology of ADHD.

Our findings might confirm the associations observed between ADHD and insomnia phenotypes in previous epidemiological studies,[16] or even the genomic positive correlations between both, as previously reported.[3,7,17] The effect found in our study on the association between insomnia PRS and ADHD supports the hypothesis that the genetic relationship between both is mediated by an additive effect of multiple common gene variants with small effect sizes, as well as the polygenic genetic architecture of individual phenotypes.[3,4] Our results also suggests that several polymorphisms associated with insomnia at less strict *p*-values might play a more important role in ADHD etiology than the stricter ones, since the PRS considering a less strict *p*-value threshold showed a nominal association. This might impact the difference on findings and describe the importance higher polygenic effect of insomnia genetic components on ADHD. However, a previous study based on the polygenic transmission disequilibrium test in two samples of complete trios of ADHD probands and both biological parents observed no evidence of differential transmission of polygenic liability for insomnia.[12]

In both the analyzed samples of European origin—one used as discovery (328 trios from Wales) and another considered as replication (844 trios from the IMAGE study)—even though the insomnia polygenic score suggested a risk

Table 1 Results for the association between insomnia PRS and ADHD using binary logistic regression model in a Brazilian clinical sample of children with ADHD

	N of variants	Pseudo R-squared	OR (95%CI)	*p*-value
Insomnia PRS				
P_T 5e-8	51	0.001	1.085 (0.913–1.290)	0.352
P_T 5e-6	205	0.000	1.035 (0.871–1.230)	0.693
P_T 0.05	**26,853**	**0.007**	**1.228 (1.030–1.462)**	**0.022***

Abbreviations: CI, confidence interval; OR, odds ratio; PRS, polygenic risk score; P_T, p-value threshold. **Notes:** *Association was considered relevant at $p < 0.05$. After Bonferroni correction for multiple tests, p-value < 0.017.

increase, it was not significantly associated with ADHD. Differences on PRS calculation and the sample's origin might explain the difference between our results and the previously published findings.

While we used the clumping plus thresholding method, considering classical thresholds for statistical analyses in biological or genomic analyses, Lewis et al.[12] used a method based on Principal Component Analysis in Plink, summarizing several thresholds in only one score. Therefore, the number of genetic markers included in each score are not directly comparable between studies, which can justify the differences between them.

Moreover, ADHD and sleep patterns are highly impacted by environmental differences, such as socioeconomic aspects and other factors that are differently represented in higher and middle-income countries.[18,19] Therefore, considering the complex etiology of both traits, genetic and environmental components in samples from different countries might also have different magnitudes. Taking that into consideration, we believe that the insomnia polygenic liability must be further evaluated in children, and these factors should be tested.

Although our results suggest that alleles that increase the risk for insomnia also increase the risk for ADHD, caution should be taken when interpreting the results. There is no GWAS for insomnia in childhood so far, and the current evidence indicated that the heritability of sleep traits seems to change throughout the person's lifespan.[2] Then, it is plausible to hypothesize that the genetic architecture of sleep phenotypes might differ between adults and children. Moreover, the clinical sample studied here was small, which limits our statistical power to find associations, mainly regarding SNPs with small effects. Another limitation is the lack of sleep problems assessment in the sample of children with ADHD, which prevents us from testing other hypotheses, as well as exploring the relationship between sleep and ADHD in this sample as done before by others.[20,21]

On the other hand, some advantages should be highlighted. We conducted the first study evaluating the potential effect of genetic components of insomnia in Brazilian children with a well-defined clinical diagnosis of ADHD, preventing measurement errors about the disorder. Furthermore, in association studies with mixed populations, such as the present one, the population stratification may cause a bias in the findings. However, the design of genetic studies in families and case-pseucocontrols is robust against population stratification problems.

Finally, our results suggest that genetic factors associated with insomnia may play a role in the ADHD genetic etiology, with both phenotypes likely to have a shared genetic mechanism. More studies are needed to clarify the genetic architecture of the relationship between insomnia and ADHD, especially research with genome-wide approaches in children and adolescents.

Conflict of Interests

Luis Augusto Rohde has received grant or research support from, served as a consultant to, and served on the speakers' bureau of Aché, Bial, Medice, Novartis/Sandoz, Pfizer/Upjohn, and Shire/Takeda in the last three years. The ADHD and Juvenile Bipolar Disorder Outpatient Programs chaired by Dr Rohde have received unrestricted educational and research support from the following pharmaceutical companies in the last three years: Novartis/Sandoz and Shire/Takeda. Dr Rohde has received authorship royalties from Oxford Press and ArtMed. Guilherme V Polanczyk has been in the past 3 years a member of advisory board of Shire/Takeda, Medice and Aché and a speaker for Shire/Takeda, Novo Nordisk and Aché. The remaining authors declare that they have no conflict of interest.

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