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Enhanced Neurobiological Biomarker Differentiation for Attention-deficit/Hyperactivity Disorder through a Risk-Informed Design

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ABSTRACT

Background: Translation of biomarkers to clinical practice is hindered by the significant overlap in neurobiological measures between ADHD cases and controls. A risk-informed design can enhance the utility and validation of ADHD biomarkers by highlighting differences between individuals with ADHD and those without at differential risk.

Methods: Participants were 2511 children and adolescents (aged 6 to 14 years) from the Brazilian High Risk Cohort for Mental Conditions. We calculated risk for ADHD among unaffected individuals using a multivariable clinical and sociodemographic risk model. We compared measures of three proposed ADHD biomarkers (polygenic scores, subcortical volumes, and executive function) between participants with vs. without ADHD, and ADHD vs. without ADHD with a high- vs. low-risk loading for ADHD.

Results: Compared to the unaffected group, children and adolescents with ADHD had higher ADHD polygenic scores (cohen's d = .17), smaller subcortical volumes (d = -.25), and poorer executive function (d = -.22). Separating the unaffected group into low- and high-risk subgroups revealed more pronounced differences (Cohen's d = .20 to .60) and nearly doubled the overlap-free area for these three neurobiological measures between the low-risk group and the other two groups. Upon adjustment for the number of ADHD symptoms, simple ADHD vs. without ADHD differences vanished, while the risk-informed analyses remained significant.

Conclusions: Here, we demonstrate that a risk-based design increases effect sizes when comparing candidate biomarkers for ADHD. Our study provides a model that may hold promise for evaluating similar contrasts in other mental disorders and samples.

Keywords: Attention-Deficit/Hyperactivity Disorder (ADHD)**;** Neurobiological Biomarkers; Multivariable; Machine Learning

INTRODUCTION

The history of medical research documents numerous investigations of potential biological markers that might aid in the diagnosis, prognosis, and treatment of mental disorders [1]. However, it remains the case that the majority of biomarkers, including genetic and neural variants, have exhibited limited applicability in clinical settings [2]. This is no less true in the case of attention-deficit/hyperactivity disorder (ADHD), a common and impairing neurodevelopmental disorder [3-6].

Several studies have demonstrated consistent mean differences in key candidate biomarkers between children, adolescents, and adults with ADHD in comparison to their unaffected counterparts [7, 8]. These differences are evident across a spectrum of objective metrics, including polygenic scores [9], structural brain parameters [7, 10], and neuropsychological performance [11, 12]. However, similar to other neuropsychiatric conditions, extensive overlap observed between cases and controls has impeded the clinical translation of such findings [8]. For instance, in a comprehensive worldwide mega-analysis assessing variations in subcortical brain volumes between individuals with and without ADHD, effect sizes ranged from .10 to .19 for mean differences in these brain structures [7, 10]. Remarkably, this translates to an overlap of 92% to 96% between groups.

This state of affairs is due, in part, to the clash between the heterogeneous and dimensional nature of mental disorders [13]. In this way, the findings of large-scale brain-wide and genome-wide studies are inherently outlined by the binary definition of cases and controls - grouping together heterogeneous presentations of ADHD in case groups [14]. However, less attention has been paid to the heterogeneity of key overlooked characteristics among unaffected individuals (controls) beyond the lack of the disorder.

One such characteristic is symptom variability over time. Longitudinal studies comprising multiple clinical assessments of ADHD reveal that age at onset of the full-blown disorder can be quite variable, even occurring well into adolescence for a subgroup of individuals [15, 16]. Traditional cross-sectional case-control studies, blinded to the trajectories of symptoms and to the potential differences in age at onset, are largely influenced by these factors. In such analyses, some individuals who are assigned to the control group might be only months or years apart from presenting with a complete clinical syndrome.

Among the array of potential methodological refinements we might envisage, a consideration of differential disease risk among unaffected individuals emerges as a particularly promising avenue [1]. This notion stems from the observation that chronic disorders often harbor insidious pathophysiological alterations long before the phenotype becomes evident. Across various mental health disorders and risk definitions, this approach can

reveal discernible alterations in biological markers between groups characterized as high and low risk for the disease [1, 2, 8].

To this end, here we aimed to disentangle the group without ADHD based on the differential risk status of unaffected individuals to determine its impact on the neurobiological contrasts with children and adolescents with ADHD. We estimated risk using a clinical and demographic multivariable model for disease onset/persistence that we have previously validated across four independent worldwide samples (i.e., c-statistics ranging from .74 to .81), including the sample described herein [17, 18]. Notably, this model stood out as one of the few (10 of 308 models) to be deemed as having a low-risk of bias in two decades of research in precision mental health, and the only one assessing a neurodevelopmental disorder [19].

In the present study, utilizing a carefully phenotyped sample from a middle-income country, we invoked a risk-informed design to compare mean differences across three candidate biomarkers for ADHD: polygenic scores, subcortical brain volumes, and executive function. These were selected as benchmark objective measures that have been widely replicated in case-control contrasts worldwide. We hypothesized that 1) the high-risk group would have an intermediate neurobiological signature, overlapping with both individuals at low-risk and those who already present with the full-blown syndrome, and 2) neurobiological measures would overlap less between cases and the low-risk group than between cases and a general (non-risk-informed) unaffected group.

METHODS AND MATERIALS

Participants

The Brazilian High-Risk Cohort (BHRC) includes 2511 children and adolescents aged 6 to 14 years at baseline recruited from 55 state-funded schools in 2010 in Porto Alegre and São Paulo, Brazil [20] (see Supporting Information). Written consent was obtained from parents and literate participants, and the study received approval from the Ethics Committee at the University of São Paulo. The current analysis focuses solely on baseline data, precisely to resemble a typical cross-sectional design.

Measures

Clinical Assessment of ADHD and other mental disorders

The child psychiatric diagnoses was carried out using the Development and Well-being Assessment [21, 22]. The DAWBA is a structured interview administered by non-specialist interviewers, which also includes the Strengths and Difficulties Questionnaire (SDQ) [21], a 25-item questionnaire that classifies individuals into four risk groups for behavioral and emotional difficulties, and recorded verbal responses about any reported problems. The verbal and structured responses have been carefully evaluated by psychiatrists, who can confirm, refute or modify the initial computer-generated diagnosis. All the questions are closely linked to the DSM-IV diagnostic criteria, focusing on current problems that cause significant distress or social impairment. The instrument has been translated into several languages and, for the present study, the Brazilian Portuguese version [22] was applied to the biological parents of all the children included in the research, following the procedures previously reported. A total of nine certified child psychiatrists carried out the assessment procedures, all trained and closely supervised by a senior child psychiatrist with extensive experience in assessing the DAWBA. In addition, all cases in which the assessors had doubts about a specific diagnosis were escalated and discussed between two child psychiatrists until a consensus on the diagnosis was reached.

Assessment and calculation of ADHD risk loading

The risk model [17] was developed in a representative birth cohort in the UK and externally validated to other clinical and population-based samples with a c-statistic of .74 to .81, including the present sample. It is based on eight clinical and demographic risk factors/markers: male sex, lower intelligence quotient, childhood

maltreatment, diagnosis of a disruptive disorder, number of ADHD symptoms, lower socioeconomic status, mother's depression, and being raised by a single-parent (see Table S1 in the Supporting Information). Based on the unique combination of these factors, we calculated the risk loading of each participant (from 0 to 1) to manifest (among controls) or persist with (among cases) ADHD up to adulthood.

Genotyping and Polygenic Scores

We collected saliva samples using an Oragene© salivary kit, and performed genotyping with the Global Screening array (Illumina) [23]. We performed p-value-informed clumping retaining the single nucleotide variants (SNV) with the smallest p-value within a 250-kb window. We excluded SNVs with a minor allele frequency <1%, locus missingness >10%, Hardy-Weinberg equilibrium significance <.000001, and we excluded individuals with genotype missingness >10% and an estimation of identity by descent >.12. Polygenic scores (PGSs) were calculated with the PRSice V2.3.5 software [24] and derived from summary statistics of the most recent Genome-Wide Association Study of ADHD [9] using a threshold of .05 (selected based on the best predictive performance for ADHD).

Subcortical Volumes

A subsample of 750 participants underwent brain magnetic resonance imaging using a 1.5T scanner to acquire high-resolution three-dimensional T1-weighted images. These images were processed with FreeSurfer following ENIGMA protocols, with quality control procedures performed subsequently. We analyzed age- and sex-adjsuted subcortical volumes of the hippocampus, putamen, caudate nucleus, amygdala, and accumbens, reflecting the findings of recent mega-analyses [7, 10]. A confirmatory factor analysis (CFA) was carried out with the subcortical volume values to test and obtain subcortical volume scores (SV) from a one-dimensional model (Table S2 in the Supporting Information).

Executive function

Assessment of executive function (EF) was conducted by a clinical psychologist and a speech therapist through a battery of standard neuropsychological assessments [20, 25-28] (Table S3 in the Supporting Information). All dependent measures were transformed into age-adjusted t-scores before analyses. Also, to avoid

false positives induced by multiple comparisons, we employed a previously validated single dimension latent measure of executive function [29] (see Supporting Information).

Statistical Analysis

We conducted the main analyses in such a way that we could estimate the magnitude of differences achieved through pairwise comparisons of neurobiological differences between participants with and without ADHD and between ADHD cases and unaffected individuals with low and high-risk loading for developing ADHD (herein called 'a risk-enriched design'). To define the latter, we refined the group without ADHD by separating individuals into those below the 10th and above the 90th percentile for low- and high-risk groups, respectively.

Then, we fitted linear regression models to regress out the effects of sex and the 5 principal components on PGSs, the effects of intracranial volume on SV (already adjusted for sex and age), and the effects of sex on EF (already adjusted for age). Standardized residuals were then compared pairwise between groups (eg., ADHD cases vs. no ADHD, low-risk vs. high-risk) using independent samples t-tests. We calculated Cohen's d as a metric to assess the magnitude of the mean differences between groups. We calculated the percentage of non-overlap shared between density plots of all three neurobiological measures between these groups.

We carried out sensitivity analyzes to evaluate the robustness of our findings. First, we repeated the same analytical procedure while also controlling for the effect of ADHD symptoms on each measure. Afterwards, we separated the group without ADHD into equivalent extremes of symptoms (i.e., below the 10th and above the 90th percentile in the number of ADHD symptoms) to evaluate whether the proposed risk-enriched design is superior to a simpler symptom-based design to refine the unaffected group. We also conducted analyses refining the group without ADHD to remove two common comorbidities (major depression and any anxiety disorders). Finally, we conducted sensitivity analyses comparing the PGSs for other neuropsychiatric disorders across groups to evaluate whether differences in the genetic signature are specific to ADHD-related genes. All the analyses were carried out using R 4.1.3 software with packages gtsummary_1.7.2 [30], lavaan_0.6-16 [31] and overlapping 2.1 [32].

RESULTS

Sample characteristics and probability of ADHD within a risk-informed design

The final sample comprised 274 participants with and 2237 participants without ADHD. The stratified unaffected group was a subsample of individuals with low (N = 224) and high (N = 224) risk loading for ADHD. The numbers of participants who provided brain imaging data are as follows: No ADHD (without risk selection) = 638, High-risk = 64; Low-risk = 66; and ADHD = 89. The mean risk for ADHD was 5.3% for all participants without ADHD, 1.1% among those at low risk, and 23.1% among those at high risk. Further statistics and the distribution of predictor variables in the complete sample can be found in Table 1.

When the risk status is not considered, children and adolescents with ADHD have subtle neurobiological differences as compared to their unaffected peers

As expected, children with ADHD had higher PGSs for ADHD compared to those without ADHD (d = .17, 95% CI = .04 to .30, p = .009, Table 2, Figure 1), while their PGSs for other common psychiatric disorders were not significantly different (Table S4 in the Supporting Information). Around 8.2% of the group distributions of PGS did not overlap. Additionally, participants with ADHD had smaller SVs (d = -.25, 95% CI = -.47 to -.03, p = .032) than their unaffected peers, and the level of non-overlap was 13.2% (Figure 2). Lastly, their age-adjusted EF scores were lower than those of individuals without ADHD (d = -.22, 95% CI = -.35 to -.10, p < .001). The distribution curves of EF had an area without overlap of 8.6%.

A risk-informed design reveals moderate neurobiological differences between the unaffected low-risk group as compared to the unaffected high-risk and ADHD groups

Children and adolescents at low risk had lower ADHD PGSs as compared to those at high risk (d = -.20, 95% CI -.39 to -.001, p = .048) and with ADHD (d = -.20, 95% CI -.38 to -.01, p = .032, Table 3, Figure 1, Figure 3). The low-risk group was not different from the high-risk group in their PGSs for other psychiatric disorders, but had lower PGSs for educational attainment as compared to the high-risk and ADHD groups (Table S4 in the

Supplementary Information). Similarly, the low-risk group had larger SVs when compared to those at high risk (d = .51, 95% CI .18 to .89, p = .003, Figure 3) and those with ADHD (d = .60, 95% CI = .28 to .96, p < .001). The low-risk group also had better EF than the high-risk group (d = .49, 95% CI .30 to .70, p < .001, Figure 3) and than the group with ADHD (d = .48, 95% CI .29 to .68, p < .001). The degree of non-overlap between the low-risk and the high-risk groups was 11.5%, 21.6%, and 20.5% for PGSs, SVs, and EF, respectively (Figure 2). The degree of non-overlap between the low-risk and the ADHD cases was 8.7%, 23.7%, and 18.9% for PGSs, subcortical volumes and executive function, respectively.

Children and adolescents unaffected with ADHD but at high risk do not have a different neurobiological profile as compared to their peers with ADHD

Participants at high-risk for developing ADHD had no statistically significant difference in ADHD PGSs as compared to participants with ADHD (d = .01, 95% CI -.21 to .19, p = .933). Similarly, we found no detectable differences in SVs (d = .12, 95% CI -21 to .46, p = .502 - Table 3, Figure 1) or EF (d = .01, 95% CI -.18 to .19, p = .912) between these groups. The degree of non-overlap between the high-risk and the ADHD groups was 5.4%, 13.0%, and 4.1% for polygenic scores, subcortical volumes, and executive function, respectively.

Controlling for the dimensional profile of ADHD symptoms suppresses the group effects of neurobiological differences in the traditional, but not the risk-informed design

When controlling for the number of ADHD symptoms, neurobiological differences observed in the traditional ADHD vs. no ADHD analysis became smaller and non-significant for PGSs (d = -.04, 95% CI -.17 to .09, p = .541), SVs (d = .16, 95% CI -.06 to .38, p = .163), and EF scores (d = .00, 95% CI -.14 to .13, p = .979). But when the same sensitivity analyses were applied to the risk-informed design, results revealed that SVs were still significantly smaller in participants with ADHD (d = -.51, 95% CI -.87 to -.18, p < .001) and those at high risk for ADHD (d = -.45, 95% CI -.84 to -.11, p = .008) when compared to low-risk participants (Table S5 in the Supplementary Information). Similarly, the group with ADHD (d = -.24, 95% CI -.43 to -.06, p = .010) and the group at high risk for ADHD (d = -.36, 95% CI -.56 to -.17, p < .001) had lower EF scores than the low-risk group. For PGSs, differences also were only slightly attenuated by controlling for ADHD symptoms (e.g., d dropped

from .20 to .18 comparing low-risk to high-risk and ADHD), although p-values surpassed the threshold of .05. In the same direction, enriching the unaffected group based only on symptom information by defining low- and high-symptom control groups produces small, mostly not statistically significant contrasts in neurobiological comparisons that completely vanish when controlled for the risk loading information (Table S6 and Table S7 in the Supplementary Information). The results were consistent when we refined the group without ADHD removing individuals with any anxiety disorder or major depression (Table S8 in the Supplementary Information).

DISCUSSION

One lingering dilemma in the field of mental health research is the widening gap between the advances in the measurement of neurobiological features, presumably related to the pathophysiology of mental disorders, and the limited applicability of candidate biomarkers in guiding diagnosis, prognosis, and treatment in psychiatric clinical practice [1]. In the case of ADHD, a common neurodevelopmental disorder, while significant group differences have been consistently demonstrated between cases and controls for genetic signature, structural brain volumes, and neuropsychological performances, the effect sizes are small, and the groups overlap to a large extent [7, 9, 33]. The approach undertaken herein demonstrated that a risk-informed design disentangled a portion of the considerable overlap shared between groups – overlap that continues to obfuscate the translation of such findings from bench to bedside in studies using standard case/control designs.

The present results validate and extend prior findings by showing subtle distinctions in polygenic scores, subcortical volumes, and executive function among children and adolescents with ADHD compared to their unaffected peers in a population-based sample from a middle-income country [20]. This mirrors previously established results predominantly derived from high-income settings and selected populations [7, 9, 33]. The effect sizes observed, quantified as Cohen's d (.17 for PGSs, and .25 for subcortical volumes, .22 for executive function), indicated that only a small area of group distributions did not overlap, ranging from 6.2% to 13% across these measures.

In subsequent analyses, we showed that leveraging a risk-informed design unveiled more pronounced distinctions, with effect sizes of a magnitude of .20 for PGSs, .50 for subcortical volumes, and .48 for executive function, when comparing unaffected children and adolescents with high vs. low-risk loading for ADHD. Interestingly, this approach nearly doubled the overlap-free area when considering these contrasts between the low-risk and the other two groups. Particularly noteworthy was the absence of discernible neurobiological differences between the ADHD group and the high-risk group, suggesting that the resulting phenotype (i.e., symptoms and impairment) might be determined either by other unmeasured biological features or by environmental factors, such as demand and stress. Another plausible interpretation is that these unaffected high-risk individuals might harbor resilience factors, a facet often overlooked in mental health scientific research [34]. Nevertheless, some caution is required to avoid overinterpreting non-statistically significant differences, considering that our study was underpowered to identify very small effects typical of biomarkers in Psychiatry.

Existing research has found that individuals at risk for mental health disorders can exhibit

neurobiological traits akin to those with established conditions. For example, Van Rooji et al. [35] showed that unaffected siblings of adolescents with ADHD displayed diminished brain activation in frontostriatal and fronto-parietal networks during response inhibition tasks compared to healthy controls. Structural and functional brain changes have also been observed in unaffected direct relatives of individuals with other mental disorders, such as depression [36], anxiety [37], and psychosis [38]. Alternative approaches to identifying at-risk individuals have utilized polygenic scores as the defining variable [39, 40, 41].

Here we employ a multivariable, empirical definition of risk. Unlike the family history approach, a clinical/demographic composite score is not limited to a specific subset of the population (i.e., relatives of affected individuals) and is likely more effective in identifying low-risk individuals, which is particularly relevant to multifactorial, polygenic disorders. Furthermore, using the composite score reveals a much steeper risk gradient than relying solely on polygenic scores.

The present study design also converges with the contemporary discussion in psychiatry nosology concerning dimensional versus categorical definitions of mental disorders [42]. Previous research has indicated that neurobiological features do not neatly align with specific thresholds of disease definition; instead, they exhibit a continuous relationship with the severity of symptoms [43]. Surprisingly, our analysis revealed that when controlling for the number of ADHD symptoms, group effects vanished in the traditional analyses but remained significant in the comparisons between low-risk and the other two groups. Further, we showed that refining the unaffected group based only on symptom-based information was much less informative than using a risk-informed design.

Our study does have certain limitations that warrant consideration. First, our definition of risk loading was based on a limited set of variables that cannot capture the full range of known determinants and predictors of ADHD. However, previous data show that adding three known ADHD predictors (prematurity, family history, and ADHD polygenic scores) did not improve the model's performance, which suggests that unmeasured biological risk might be encompassed in the combination of clinical and demographic factors [18]. However, future approaches with additional risk markers could achieve even larger effects between risk-informed control groups. Second, our sample size is small in comparison to large international neuroimaging and genetic consortia. Consequently, we opted to focus on one-dimensional variables rather than conducting exploratory analyses to identify specific alterations in brain volumes, genetic signatures, or neuropsychological features that could be unique to a high-risk designation but might fail to replicate in external samples. Third, most individuals included

in GWAS are from European ancestry, which may have reduced the observed effects in the genetic architecture of the different risk groups and ADHD case groups in this particular study. Finally, the differences between these groups are small and, in detailed comparisons, almost non-existent, showing that current methods may not be sufficiently robust. Therefore, it is essential that future research explores alternatives that can identify new markers or methodologies, especially focused on distinctions between the extremes of the risk spectrum, to increase the reliability and effectiveness of using biomarkers for ADHD.

CONCLUSION

In conclusion, employing a risk-informed design powerfully revealed larger effect sizes when distinguishing key candidate biomarkers between individuals affected by ADHD and unaffected but with different risk loadings. Our findings shed new light on the considerable overlap observed across comparison groups in traditional case/control analyses, by underscoring the importance of considering multivariable risk profiles to move beyond traditional group definitions. Moreover, the present study highlights the need for further exploration into the role of resilience factors and the dynamic nature of symptomatology over time. Future research might further integrate multivariable-defined risk information to stratify control groups according to their risk spectrum, to better understand the specific neurobiology involved in disease onset, remission and resilience throughout development.

ETHICAL CONSIDERATIONS

The authors assert that all procedures contributing to this study comply with the ethical standards and are approved by the National Research Ethics Commission (Comissão Nacional de Ética em Pesquisa), the University of São Paulo and the Federal University of Rio Grande do Sul ethics committees. The authors assert that all procedures contributing to this study also comply with the Helsinki Declaration of 1975, as revised in 2008.

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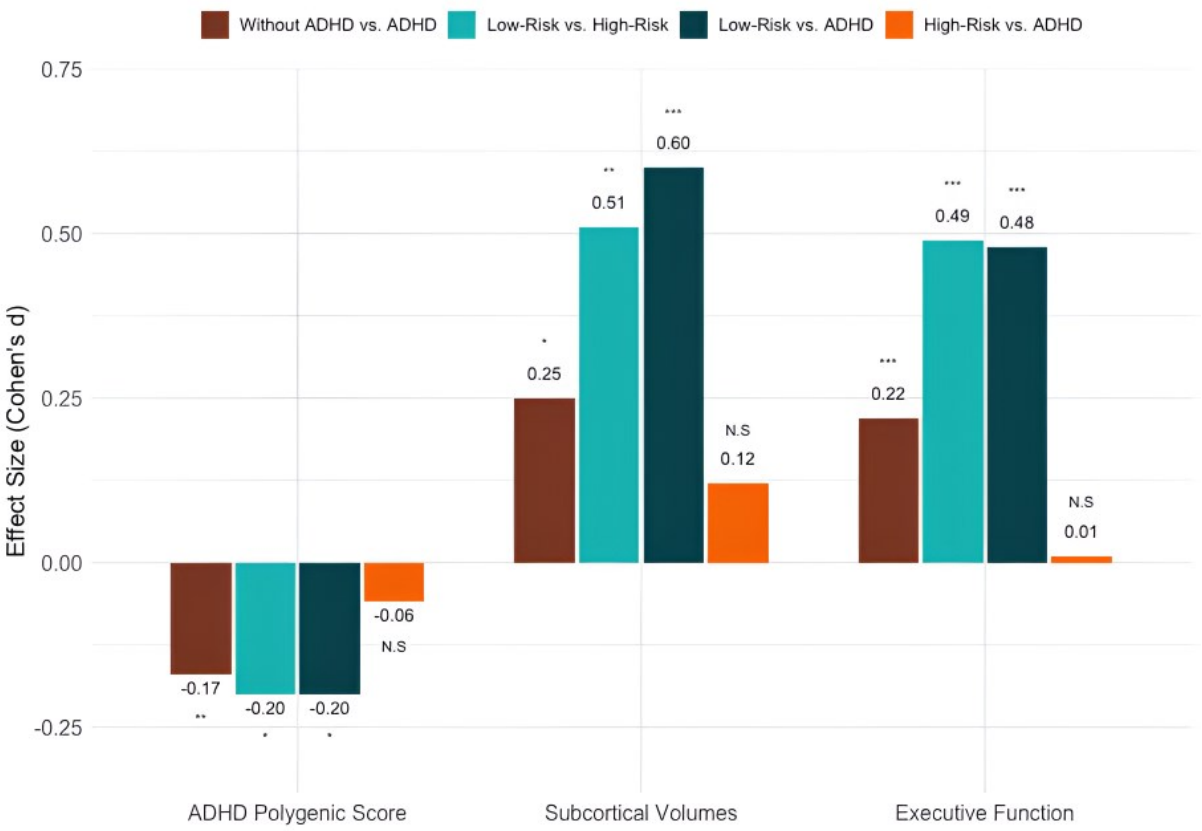
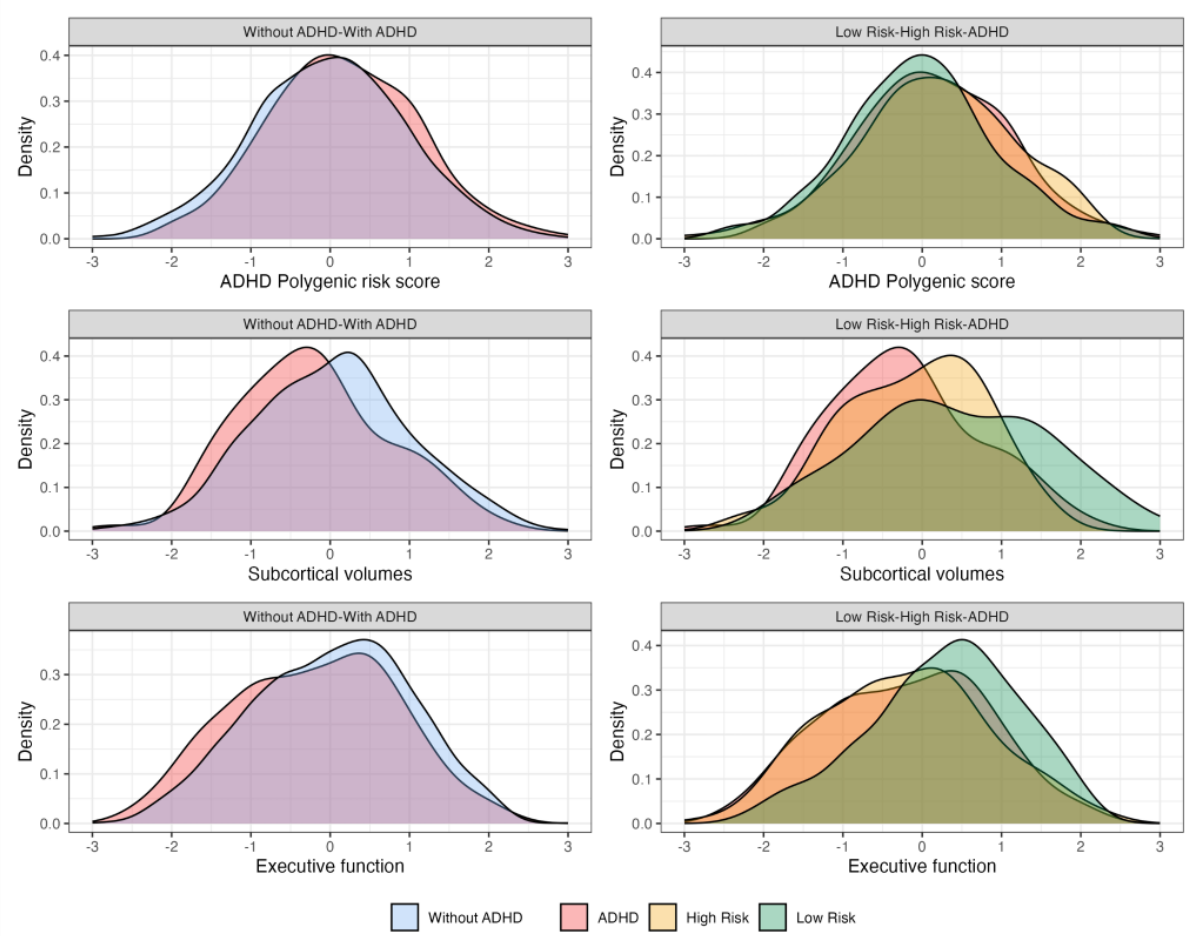
FIGURE/TABLE LEGENDS

Figure 1. Effect sizes of comparison groups in subcortical volumes, executive function, and ADHD Polygenic Score



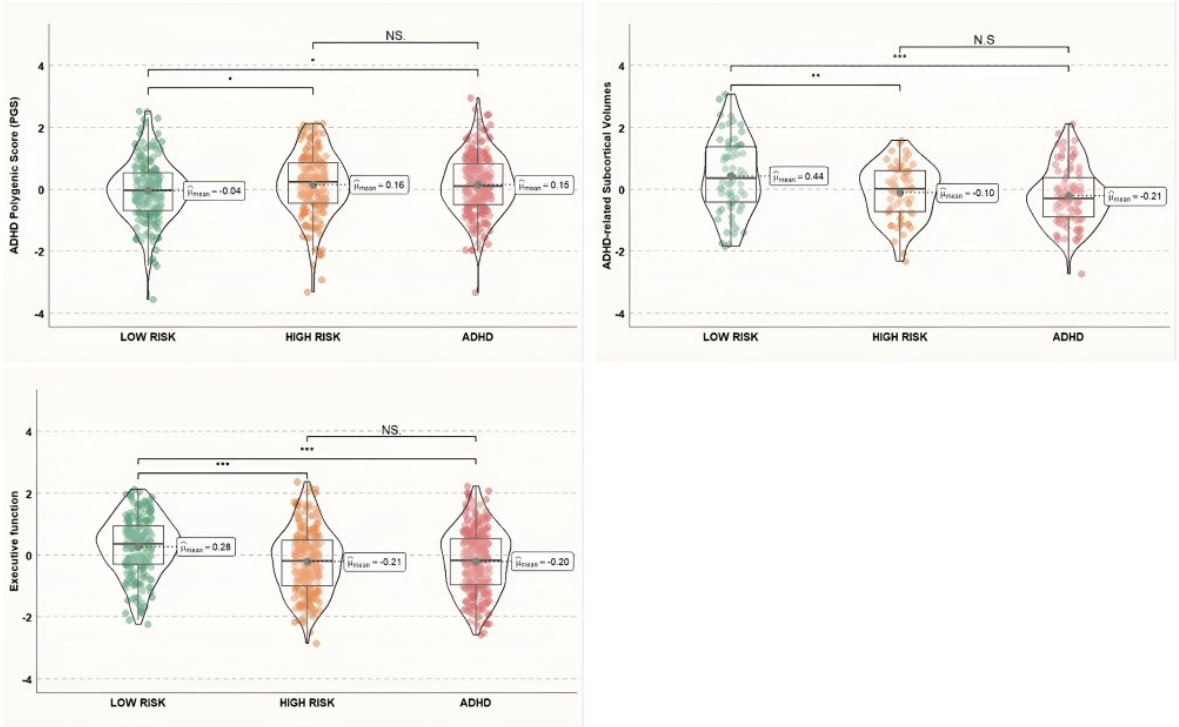
This bar chart displays the effect sizes (Cohen's d) and statistical significance of comparisons between groups. Colors represent contrasts that generated the d statistic: brown bars for ADHD vs. not ADHD, green for the comparison between low-risk and high-risk, moss green for the comparison between low-risk and individuals with ADHD, and orange for the comparison between high-risk and individuals with ADHD. The labels on the x-axis indicate the measures evaluated. Statistical significance levels are indicated as follows: < 0.05 is represented by"*", < 0.01 by "**", < 0.001 by "***", and non-significant results by "N.S.".

Figure 2. Density plots of subcortical volumes, executive function, and polygenic scores across comparison groups



The figure consists of a series of six overlapping density distributions for the three groups (unaffected low-risk, unaffected high-risk, and ADHD) across the z-scores of ADHD Polygenic Score, Subcortical Volumes and Executive Function. In the left-hand column, the comparisons are between individuals without ADHD (in blue) and individuals with ADHD (in red). In the right-hand column, the comparison is threefold, including low-risk individuals (in green), high-risk individuals (in yellow) and individuals with ADHD (in red).

Figure 3. Boxplots comparing the low-risk, high-risk and ADHD groups in the three measures evaluated in this study (polygenic score, subcortical volume and executive function)



The figure is made up of three boxplots and shows the comparison of the polygenic score for Attention Deficit Hyperactivity Disorder (ADHD), subcortical volumes related to ADHD and executive function between three groups: individuals at low risk for ADHD, individuals at high risk for ADHD and individuals diagnosed with ADHD. Statistical significance levels are indicated as follows: < 0.05 is represented by"*", < 0.01 by "**", < 0.001 by "***", and non-significant results by "N.S.".

TABLES

Table 1. Frequency and distribution of demographic and clinical predictors of the sample

		WITHOUT ADHD		WITH ADHD
	All	**Low Risk**	**High Risk**	**ADHD**
	(n = 2237)	**(n = 224)**	**(n = 224)**	**(n = 274)**
Female sex	1,038 (46.4%)	156 (69.6%)	78 (34.8%)	98 (35.8%)
Age[a]				
6 to 8 years old	486 (21.7%)	51 (22.8%)	40 (17.9%)	71 (25.9%)
9 to 11 years old	1,134 (50.7%)	124 (55.4%)	125 (55.8%)	141 (51.5%)
12 to 14 years old	617 (27.6%)	49 (21.9%)	59 (26.3%)	62 (22.6%)
Number of ADHD symptoms[1]	0	0	4 (3, 7)	10 (7, 13)
Intelligence quotient[2]	102.1 (15.7)	116.8 (13.8)	95.8 (15.7)	98.3 (16.7)
Maltreatment				
None	917 (41.0%)	172 (76.8%)	27 (12.1%)	56 (20.4%)
Probable	701 (31.3%)	33 (14.7%)	85 (37.9%)	77 (28.1%)
Severe	619 (27.7%)	19 (8.5%)	112 (50.0%)	141 (51.5%)
Depression of the mother	300 (13.4%)	2 (0.9%)	87 (38.8%)	60 (21.9%)
Socioeconomic status				
Upper	370 (16.5%)	163 (72.8%)	16 (7.1%)	28 (10.2%)
Middle	279 (12.5%)	4 (1.8%)	46 (20.5%)	29 (10.6%)
Lower	1,588 (71.0%)	57 (25.4%)	162 (72.3%)	217 (79.2%)
ODD or CD	79 (3.5%)	0 (0.0a%)	46 (20.5%)	83 (30.3%)
Single parent family	1,060 (47.4%)	36 (16.1%)	148 (66.1%)	145 (52.9%)
Risk loading[2]	**5.3% (7.3%)**	**1.1% (0.2%)**	**23.1% (10.6%)**	**59.0% (34.7%)**
Major depression	59 (2.6%)	1 (0.4%)	25 (11.2%)	14 (5.1%)
Any Anxiety Disorder	131 (5.9%)	10 (4.5%)	21 (9.4%)	28 (10.2%)

[a]Age is not entered in the predictor model since it was originally developed in a birth cohort with homogeneous age within participants (i.e., age was invariant). [1] Median (Interquartile Range) [2] Mean (Standard Deviation)
ADHD Attention-deficit/hyperactivity disorder *ODD* Oppositional Defiant Disorder *CD* Conduct Disorder. Major depression (Clinical rating, DSM-IV). Any Anxiety Disorder (Clinical rating, DSM-IV), which includes agoraphobia, generalized anxiety disorder, social phobia, specific phobia.

Table 2. Effect sizes of comparisons of subcortical volumes, executive function and ADHD polygenic score between participants with and without ADHD in a traditional cross-sectional design

Measure	Comparison	Sample size	Cohen's d [95% CI]	p-value
ADHD Polygenic score	With ADHD	2188	.17 [.04 to .30]	.009**
Subcortical volumes	vs.	727	-.25 [-.47 to -.03]	.032*
Executive function	Without ADHD	2398	-.22 [-.35 to -.10]	< .001 ***

Significance level: <.001 = ***; <0.01 = **; < 0.05 = *
ADHD = Attention-deficit/hyperactivity disorder; CI = Confidence interval

Table 3. Effect sizes of comparisons of subcortical volumes, executive function and ADHD polygenic scores between individuals with and without ADHD in a risk-informed design

Measure	Group comparison	Sample size	Effect size (Cohen's d [95% CI])	p-value
ADHD Polygenic score	Low-Risk vs. High-Risk	390	-.20 [-.39 to -.001]	.048*
	Low-Risk vs. ADHD	442	-.20 [-.38 to -.01]	.032*
	High-Risk vs. ADHD	440	.01 [-.21 to .19]	.933
Subcortical volumes	Low-Risk vs. High-Risk	128	.51 [.18 to .89]	.003**
	Low-Risk vs. ADHD	153	.60 [.28 to .96]	< .001***
	High-Risk vs. ADHD	151	.12 [-.21 to .46]	.502
Executive function	Low-Risk vs. High-Risk	433	.49 [.30 to .70]	< .001***
	Low-Risk vs. ADHD	484	.48 [.29 to .68]	< .001***
	High-Risk vs. ADHD	485	.01 [-.18 to .19]	.912

Significance level: < 0.05 = *, <0.01 = **; <.001 = ***;
ADHD = Attention-deficit/hyperactivity disorder; CI = Confidence interval