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On the role of non-shared environment for executive functioning in ADHD : a twin-differences design study

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Abstract

Introduction: The study of differences between monozygotic (MZ) twin pairs with respect to ADHD may provide novel leads to disentangle the environmental contribution driving its phenotypes.

Objectives: To examine non-shared environmental influences on executive function in dimensionally defined ADHD.

Methods: This study included 27 MZ twin pairs (7 female) aged 11-20 years being moderately to substantially discordant for ADHD traits as assessed by the Attention Problem (AP) scale of the Child Behavior Checklist/Adult Behavior Checklist. The twins completed the Wisconsin Card Sorting Test (WCST) for cognitive flexibility and Tower Test (TT) for foresighted planning. Two statistical approaches were used to analyze the data. First, correlations between ADHD trait intra-pair differences and WCST and TT scores were calculated. Second, the significance of those intra-pair differences on WCST and TT, using ADHD as categorical variable in clinically discordant pairs, was tested.

Results: Both analyzing strategies revealed a link between ADHD on one hand, and foresighted planning and inhibitory control on the other hand mediated by non-shared environmental factors. The first statistical approach yielded positive correlations between intra-pairs differences on the AP scale and intra-pair differences on two subscales of the TT: Total Rule Violation ($r_s=.41$) and Rule-Violation-Per-Item-Ratio ($r_s=.38$). Findings in categorically discordant pairs were consistent, showing within-pair differences on the same subtests ($z=1.63$, $p=.05$, one-tailed and $z=-1.60$, $p=.05$, one-tailed).

Conclusions: Findings confirm previous research suggesting ADHD to be a quantitative extreme on a continuum with executive functions being a cognitive marker of ADHD traits. Non-shared environmental factors appear to influence planning skills and inhibitory control.

Key words

Neurodevelopmental disorders, NDD, attention deficit hyperactivity disorder, neuropsychology, neuropsychiatry, discordant twin pair design, autism spectrum disorders, impulsivity, inhibition, concordance, discordance, monozygotic twin pairs

Introduction

Attention-Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized by motor overactivity, inattention and impulsivity. The defining criteria for ADHD outlined by the recently published 5th edition of the Diagnostic and Statistical Manual (DSM-5; American Psychiatric Association, 2013) include descriptions of 18 symptoms in two affected behavioral domains (inattention and hyperactivity/impulsivity). Three different subtypes are defined (combined, predominantly inattentive, and predominantly hyperactive/impulsive) as well as three severity levels (mild, moderate and severe). ADHD is one of the most common developmental disorders, with a prevalence of 5-7% in children/adolescents and 2.5% in adults. The diagnose is 2-3 times more common in males than females (Polanczyk et al.; 2007; Simon et al., 2009; Wilcutt, 2012). Traditionally, ADHD has been described as a childhood disorder. However, ADHD symptoms and related functional disabilities persist into adulthood in a substantial proportion of individuals, although the symptoms might change with age. A triple pathway model of neuropsychological functioning in ADHD is favored, focusing on three areas of deficits; central inhibitory control, delay aversion and temporal processing (Sonuga-Barke et al., 2010). The etiology of ADHD remains puzzling (Tripp and Wickens, 2009). No single risk factor causes ADHD; rather both genetic and non-genetic factors and their interplay contribute. Research examining molecular genetic factors in ADHD suggests an overlap with other neurodevelopmental disabilities, such as autism spectrum disorder and dyslexia (Ronald and Hoekstra, 2011; Smalley et al., 2005; Thaper et al., 2013).

Genetic and environmental contributions to ADHD

Approximately 70% of impulsive-hyperactive and inattentive symptom variation in ADHD is explained by genetic factors (Nikolas and Burt, 2010). In a large sample of Swedish twins (N=16,366), genetic and environmental causes for ADHD were examined at extreme levels compared to sub-threshold levels of symptom severity (Larsson et al., 2012). A heritability of 60%

was found in both groups, indicating that ADHD is best described as an extreme end of a quantitative trait. Several twin studies based on the Twin Early Development Study (TEDS) have focused on the overlap between inattentive and hyperactivity/impulsive ADHD symptoms. Greven and colleagues (2011) examined monozygotic (MZ) and dizygotic (DZ) twin pair correlations for the two main ADHD symptom domains. They found a MZ correlation of .88 (DZ $r = .48$) for hyperactive-impulsive symptoms, and a MZ correlation of .79 (DZ $r = .37$) for the inattentive symptoms. A genetic correlation between the two domains of .55 was reported, indicating that the two domains are substantially influenced by the same genes, but also that the two domains show large unique genetic effects.

Non-shared environmental influences

Although largely genetically determined, both shared and non-shared environmental factors also impact ADHD etiology (Burt et al., 2012). Non-shared environment is non-genetic factors that result in differences between individuals growing up in the same family. Examples of potential non-shared environmental factors are epigenetic alterations, infections, nutrition, toxic hazards, neurological injuries, parental treatment, school environments, and peer relations. The most frequently reported environmental risk factors in ADHD, both shared and non-shared, are pregnancy-related such as maternal diabetes (Nomura et al., 2012), viral or bacterial infections (Mann and McDermott, 2011), preterm birth below 26 weeks of pregnancy (Johnson et al., 2010) and low birth-weight (Groen-Blokhus et al., 2011; Hultman et al., 2007).

Executive functioning (EF)

Neuropsychological studies have confirmed that executive malfunction is a core problem in ADHD, with central behavioral inhibition as the core underlying difficulty (Barkley, 1997; 2010). Alterations in the frontal lobes, caudate nucleus, and cerebellar vermis are likely neurobiological correlates (Tripp and Wickens, 2009). EF governs goal-directed cognitions including planning,

working memory, problem solving, mental flexibility, and adequate decision making to adjust to an ever changing environment. In everyday life, EF enables independent and purposive behavior in all areas of life, including academic achievements, and social relationships (Jurado and Rosselli, 2007). EFs can be conceptualized into four components; volition, planning, purposive action and effective performance (Muriel et al 2004).

Two widely used measures of EF are the Wisconsin Card Sorting Test (WCST; Heaton et al., 1993; Steinmetz and Houssemand, 2011) and the Tower Test (TT) from D-KEFS. The WCST measures cognitive flexibility and set shifting, and the TT measures foresighted planning (Dean et al., 2001; Zook et al., 2004). Discriminant validity has been comprehensively analyzed for both tests. Children with ADHD show poorer performance on these tests compared to controls (Romine et al., 2004; Sergeant et al., 2012). Classic twin designs of EF in non-clinical samples of MZ and DZ twin pairs have overall showed no or limited genetic effect on WCST-performance (Chou et al., 2009). However, studies have revealed gender differences for the relative contribution of genes and environment on the abilities measured in WCST. A greater impact of genetic influence has been found in female adolescents compared to males, although with an overall increasing influence of genetic factors on frontal executive functioning during adolescence (Anokhin et al., 2003). Environmental factors that have been suggested to influence EF include parental educational level and socioeconomic status (Ardila et al., 2005; Sarsour et al., 2011).

Twin differences design

A powerful application of the twin design is the study of differences in a phenotype within MZ twin pairs. Since MZ twins have identical nucleotide chromosomal DNA sequence, except for errors in DNA replication, which remain a minority, all differences within MZ twin pairs are ascribed to environmental factors and/or measurement errors (Plomin and Daniels, 1987). As a result, all differences within MZ twin pairs being discordant for a trait or a disorder could be of

etiologically. The design provides unique means for a maximum control of potentially confounding factors. Though, only a limited number of studies have been published on ADHD discordant MZ twin pairs to date (Castellanos et al., 2003; Lehn et al., 2007; Pearsall-Jones et al., 2008, 2009; Sharp et al., 2003; Van 't Ent et al., 2009). Results from these studies report differences in brain anatomy, i.e. reduced caudate nucleus and prefrontal lobe volumes in cases (Castellanos et al., 2003), and the identification of environmental risk factors such as low birth weight, delayed motor development, and being born the second twin in the pair (Lehn et al., 2007). Furthermore, results from functional Magnetic Resonance Imaging (fMRI) in twins pairs discordant and concordant for ADHD indicate that attention problems caused by genetic versus non-shared environmental factors, affect the brain in different ways. On one hand, attention problems attributable to genetic factors were shown to be associated with decreased activation in the frontal brain regions and parietal brain lobes during executive tasks. On the other hand, attention problems of non-genetic origin correlated with decreased activation in the same brain regions during only one task (Van't Ent et al., 2009). In summary, MZ discordant twin pair studies of ADHD have been of limited scope and relatively small, partly overlapping, samples (N < 20 MZ discordant pairs).

In this study, we address the origins of executive malfunctioning in ADHD. More precisely, the aim of this study is to investigate non-shared environmental effects on cognitive flexibility and planning skills in MZ twin pairs being discordant for ADHD traits. We hypothesize that intra-pair difference in ADHD traits are correlated to the intra-pair differences on EF measures, both in pairs with minor and large differences in ADHD traits. Further, in categorically (clinically) discordant pairs we hypothesize that the twin without ADHD will outperform their ADHD diagnosed co-twin on EF measures.

Method

Procedure

The participants were recruited from the ongoing research project Roots of Autism and ADHD Twin Study Sweden (RATSS). The RATSS study is led by the Center for Neurodevelopmental Disorders at Karolinska Institutet (KIND) in close collaboration with a multitude of partners, and is tightly connected to the Child and Adolescent Twin Study in Sweden (CATSS) and the Swedish Twin Registry (Anckarsäter et al., 2011). RATSS collects and analyzes data on multiple levels (e.g., psychopathology, medical history, dysmorphology, neuroimaging, environment, and (epi)genetics) including neuropsychology, from MZ and DZ twin pairs with aim of finding new leads for causal pathways and treatment options in ADHD and ASD.

The twin pairs included in the study were mainly identified via the Swedish Twin registry and recruited from all over Sweden. The families were first contacted via mail and then via phone. Twins and their parents were either assessed at the Astrid Lindgren's Children Hospital or at KIND, in Stockholm. Questionnaires were sent to the families for completion ahead of their visit. For families not living in the Stockholm area, travel and accommodation were arranged by the study coordinator. The twins were examined separately, while their parents were interviewed. Each family was assessed by three licensed psychologists trained on the different instruments. The order of tests was randomly switched within and between the pairs. The twins were assessed by neuropsychiatrists and clinical geneticists, for differential and comorbid diagnoses. Total assessment duration was seven hours including a longer lunch break and several shorter intervals. Best estimate psychiatric clinical consensus diagnoses based on all gathered information was made by the team of psychologists and coordinated with the medical doctors.

Sample

In the present study, 27 MZ twin pairs, out of a sample of 41 examined twin pairs within the RATSS study, were selected as filling the inclusion criteria. The selection criteria were DNA-test

showing monozygosity, complete data on the measures of interest and a moderate to substantial intra-pair difference (≥ 1 point difference) for ADHD traits. ADHD traits were assessed by the Attention Problems (AP) scale of the parent-reported Children Behavior Checklist (CBCL/6-18) or the Adult Behavior Checklist (ABCL/18-59) (Achenbach and Rescorla, 2001; 2003), and by the self-rated AP scale of Youth Self Report (YSR/11-18) or Adult Self Report (ASR/18-59). The demographics and clinical measures for the sample are shown in Table 1.

About here Table 1

The age of the twins ranged from 11 to 20 years, with a mean age of 15.2 years (SD 3.0), and a sex ratio of 20:7 (male:female). The presence of a clinical ADHD diagnosis was evaluated using expert consensus based on DSM-IV-TR (even fulfilling DSM-5) criteria corroborated by information from the Kiddie-Schedule Affective Disorders and Schizophrenia (K-SADS; parent report) (Kaufman et al, 1997) or DSM-IV checklists, medical history taking, and medical records. Eighteen participants were diagnosed with ADHD (combined N=7; inattentive N=6; not otherwise specified N=5). The mean intra-pair differences on the CBCL/ABCL AP scale was 3.4 (SD 2.2, range 1-11). For analyses of sex differences, the twin/co-twin sample was subdivided into male and female participants (mean age was 15.1 years in both groups). Other confounders such as age and birth order were controlled for by partial correlation analyses. Moreover, three out of the 27 twin pairs had been diagnosed with twin-to-twin transfusion syndrome (TTTS). TTTS is a complication of monochromic twin pregnancies and is the result of inter-twin blood transfusion through placental vascular anastomoses. The syndrome is characterized by a gradual shift of blood volume from the donor twin to the recipient twin through placental vascular connections (De Paepe and Luks, 2013). In order to exclude risk for bias owing to TTTS, analyses were performed both with and without those pairs. For all pairs included in this study, twin zygosity was determined on a panel of 48 SNPs in saliva samples. At time for assessment, 13 out of the 54 participants received medication for ADHD symptoms (in five pairs both twins were medicated).

IQ was assessed with the General Ability Index (GAI) of the Wechsler Intelligence Scale for Children 4th Edition (WISC-IV) or Wechsler Intelligence Scale for Adults 4th Edition (WAIS-IV) (Wechsler, 2004; 2008). GAI includes three verbal comprehension subtests (Vocabulary, Comprehension, and Similarities) and three Perceptual Reasoning subtests (Block design, Matrix reasoning, and Picture concepts) of the comprehensive WISC-IV/WAIS-IV. Comparisons of intellectual abilities, showed no significant IQ differences between the higher ADHD traits twins and lower ADHD traits twins ($t(26)=-1.434, p=>.05$).

From the total twin-co-twin sample of 27 pairs, four pairs were clinically/categorically discordant for ADHD, i.e. one twin fulfilling the DSM-IV-TR criteria for ADHD diagnoses (one predominantly hyperactivity/impulsivity, and ADHD not otherwise specified) and the co-twin not fulfilling diagnostic criteria for ADHD. Two male and two female pairs were included in this sample and the mean age was 12.75 years (SD 1.0, range 12-14). Mean IQ (GAI) was 106.75 (SD 9.74, range 100-121) for the twins fulfilling ADHD diagnoses, and the mean IQ was 106.75 (SD 11.32, range 95-121) for their four co-twins. In two of the four pairs, the first born twin was diagnosed with ADHD, and in two pairs was the second born twin was diagnosed with ADHD. Analyses of birth weight showed no significant differences between the ADHD affected twins and their co-twins ($t(3)=2.081, p>.05$). In all four pairs, both twins visited to the same school, and were included in mainstream pedagogical settings without extra assistance. For two of the twins with ADHD diagnoses the parents reported postnatal complications (i.e. low energy after delivery, treatment in heating incubator and feeding problems) not reported in their co-twins. A history of more severe somatic problems were reported in three of the twins with ADHD diagnoses (i.e. diabetes type-I, frequent cold and ear infections in childhood and nystagmus caused by virus) and not reported in their co-twins. Two of the twins with ADHD diagnoses were medicated for their ADHD symptoms at the time for assessment.

Measures

ADHD traits

For participants aged up to 18 years old, the parents completed the CBCL, and for older participants, the parents filled-out the ABCL (Achenbach and Rescorla, 2001; 2003). The CBCL and ABCL comprise demographic information, description of functioning and ratings of behavioral, emotional and social problems. Syndrome scales are derived from CBCL/ABCL, one of them being the attention problem (AP) scale for assessment of ADHD related symptoms. The same versions of these scales (child or adult) were collected for both twins in all pairs. The AP scale was used as a measure of ADHD traits and to classify twins with higher and lower ADHD symptom load. The AP scales consist of items that are considered satisfactorily consistent with DSM criteria for ADHD. The AP scale includes 10 (CBCL) or 16 items (ABCL) rated on a 0-2 scale (0="not true", 1="somewhat true or sometimes true", 2="very true or often true"), with a maximum score of 20 on CBCL and 32 on ABCL. Example of items are "Fails to finish things that he/she starts", "Can't concentrate, can't pay attention for long", "Can't sit still, restless, or hyperactive" and "Impulsive or act without thinking". The AP scale has shown a moderate to high agreement with clinical ADHD diagnosis in previous studies, and the scale has been used in a large number of studies of ADHD (Achenbach and Rescorla, 2001; Derks et al., 2006; Galeria et al., 2013; Lehn et al., 2007; Van 't Ent et al., 2009). *Executive functioning*

The WCST computer version CV 4 was used for assessment planning skills, use of feedback from the environment and shifting of strategies, i.e. measuring cognitive flexibility and tap set shifting (Heaton et al., 1993). The test also measure abilities of inhibition, set maintenance, concept formation and rules detection (Jurado and Roselli, 2007). In the WCST, the examinee is shown cards that differ in shapes, colors and numbers, and is told to match the cards, but not how to do the matching. After each match the examinee is told by the computer whether the answer is correct or not. The test produces nine performance indexes. The most used and sensitive variable for

executive malfunction (as an indicator of impairments in cognitive flexibility) is the measure of perseveration, which is obtained by counting the number of times a subject sorts according to a previously correct principle, despite negative feedback (Heaton et al., 1993). In this study, all process measures related to perservation were included (Total errors, Perseverative responses, Perseverative errors). Validity studies of the WCST have shown that children with ADHD consistently show poorer performance on WCST compared to controls (Romine et al., 2004). Sergeant and colleagues (2002) found that the WCST significantly differentiated between ADHD children and controls in 17 out of 26 studies.

The Tower Test (TT) is a cognitive test included in the Delis_Kaplan Exeutive Function System (D-KEFS; Dean et al., 2001). Itmeasures spatial planning, rule learning, inhibition of impulsive and perseverative responding, and the ability to establish and maintain an instructional set. The TT is composed of a series of nine items, each one more difficult than the previous. The examinee is shown a picture of a tower, and the task is to move disks of various sizes across three pegs until the target tower is built, using as few numbers of moves as possible. The TT provides an overall sum score, and five process measures. Research has shown some TT variables, namely total score and rule violation (total violations, rule-violations-per-item-ratio), to discriminate between ADHD and typically developing controls (Seargent et al., 2002). Thus, the latter TT performance measures were assessed in this study. Reliability studies for the TT have shown a moderate to high internal consistency, ranging from $r_{tt} = .43$ to $.84$ for different ages (Dean et al., 2001).

Discriminant validity for TT regarding ADHD has been assessed in at least seven studies and in five of these the total score and/or rule violation indexes discriminated ADHD from controls (Aman et al., 1998; Klorman et al., 1999; Pennington et al., 1993; Weyandt et al., 1994, Wiers et al. 1998).

Statistical analyses

Two approaches were used for data analyses. In the first analyses, ADHD was treated as a

continuous trait, and in the second approach, ADHD was treated as a categorical clinical variable. Frequency distributions, skewedness and normality were examined for each independent and dependent variable. Since skewedness (>1.0) was found in several of the process measures, nonparametric statistics were used for comparisons and correlations. For the ADHD trait analysis, within-pair differences were calculated using a simple difference design (Turkheimer and Waldron, 2000), i.e. correlating the size of the intra-pair difference for each pair on the AP scale with the size of the intra-pair difference for EF measure. Spearman's rho statistic was applied to calculate the correlation coefficient. One-tailed probabilities were used because the intra-pairs differences in ADHD traits were hypothesized to correlate positively with intra-pairs differences in executive malfunction. Outliers were identified as >2.5 SD from the mean for intra-pair differences on each variable. Calculations controlling for the outliers were made to confirm that the outliers were not driving the results. Furthermore, analyses of potential confounders as sex, age, IQ, medication for ADHD symptoms, twin-to-twin-transfusion syndrome and birth order were conducted. Sex differences were examined by separate calculations of correlations coefficients in the group of males and females. The correlation coefficients were thereafter converted into z-scores using Fisher's r-z transformation, and compared between the two groups (males and females) by using formula 2.8.5 from Cohen and Cohen (Preacher, 2002). The influence of age was controlled for by partial correlation analyses. To control for TTTS, parent reported cases were identified and excluded ($n=3$ pairs) to investigate the effect on the results. A potential effect of medication for ADHD symptoms was checked by observing whether the exclusion of pairs where only one of the twins was on medication would alter the results. For analyses of ADHD as a categorical clinical variable, intra-pair differences were calculated for each separate outcome measure with Wilcoxon Signed rank test. Again, owing to a directed hypothesis, one-tailed probabilities were used for these analyses, since the more affected twin was expected to show more executive malfunctions than the less affected co-twin.

Results

ADHD traits

Descriptive results for male and female twin pairs on the WCST and TT, and analyses using ADHD traits as a continuous variable, are presented in Tables 2 and 3.

About here Table 2

About here Table 3

Analyses of all twin pairs yielded significant positive correlations between intra-pairs differences on the AP scale and intra-pair differences on two subscales of the TT: *Total Rule Violation* ($r_s=.41, p=.017$, one-tailed; see Figure 1) and *Rule-Violation-Per-Item-Ratio* ($r_s=.38, p=.03$, one-tailed). Intra-pair differences on the TT subscale *Time-per-move-ratio* also correlated positively with intra-pair differences on the AP scale ($r_s=.22$), but did not reach significance ($p=.14$, one-tailed). No associations were identified for intra-pair ADHD trait differences and WCST performance ($r<.09, p>.33$). Next, we examined the effect of sex, age, outliers, medication for ADHD symptoms, TTTS and birth order on the pattern of the aforementioned correlational findings. When analyzing males and females separately, the results in the male twin pairs resembled the correlations found in the total sample, showing positive correlations between intra-pairs differences for ADHD traits and the Tower subscales *Total Rule Violation* ($r_s=.49, p=.01$, one-tailed) and *Rule-Violation-Per-Item-Ratio* ($r_s=.46, p=.02$, one-tailed), but not the WCST. However, in female pairs, high positive correlations were found between intra-pair differences for ADHD traits and three subscales on WCST, *Total errors* ($r_s=.79, p=.02$, one-tailed), *Perseverative responses* ($r_s=.73, p=.03$, one-tailed), but not for the TT measures. Comparisons of correlation coefficients between male and female twin-pairs showed significant differences for WCST total errors ($z=-2.37, p=.02$, two-tailed). No significant differences were found on the EF measures when comparing the twins born first and second ($p >.5$). Neither age influences (*Total*

Rule Violation; $r_s = .45$, $p = .01$ and *Rule-Violation-Per-Item-Ratio*; $r_s = .37$, $p = .03$, one-tailed), nor the exclusion of the outliers (*Total Rule Violation*; $r_s = .39$, $p = .02$ and *Rule-Violation-Per-Item-Ratio*; $r_s = .49$, $p = .005$, one-tailed) showed an effect on the structure of the results. Other possible confounders such as IQ (GAI) (*Total Rule Violation*; $r_s = .42$, $p = .02$ and *Rule-Violation-Per-Item-Ratio*; $r_s = .46$, $p = .01$, one-tailed), TTTS (*Total Rule Violation*; $r_s = .47$, $p = .01$ and *Rule-Violation-Per-Item-Ratio*; $r_s = .43$, $p = .02$, one-tailed), and medication (*Total Rule Violation*; $r_s = .40$, $p = .03$ and *Rule-Violation-Per-Item-Ratio*; $r_s = .36$, $p = .044$, one-tailed) did neither alter the structure of the results.

About here Figure 1

Categorical/clinical ADHD

Next ADHD was analyzed as a categorical variable based on clinical diagnoses in four discordant pairs. These analyses yielded a trend for intra-pair differences for total rule violation ($z = 1.63$; $p = .051$, one-tailed) and Rule-Violation-Per-Item Ratio ($z = 1.60$; $p = .054$, one-tailed). Results from paired sample statistics with Wilcoxon Signed rank test are shown in Table 4.

About here Table 4

Discussion

We hypothesized an association between dimensionally and categorically/clinically defined ADHD phenotypes and executive malfunction in terms of lack cognitive flexibility/set shifting and foresighted planning. This prediction was partly supported, indicating an influence of non-shared environmental factors on EF. No significant effects of IQ, age, TTTS, medications for ADHD symptoms or birth order were found for these associations. Although we did not include an explicit measure of this in the study, our pattern of results might be best interpreted in terms of inhibitory control, which is an EF measured to a greater or lesser degree in all the test applied. A lack of inhibitory control appears to be an overarching cognitive limitation in ADHD (Happé et al., 2006), even if EF deficits in ADHD are generally considered heterogenous (Corbett et al., 2009). Our results support the notion of central inhibition as a core aspect of ADHD (Barkley, 1997; Happé et al., 2006; Sonuga-Barke, 2010). The results from our study MZ twin show intra-pair differences related to planning and inhibitory control, indicating an influence by non-shared environmental effects, at least in boys. In a larger perspective, these findings could be of clinical relevance in the development of treatment and habilitation programs. If the identified association is owing to changeable or reversible non-shared environmental factors, planning and inhibitory control might be areas with larger potential for change in comparison to other areas of executive functions with higher degree of genetic influence. However, it still remains unclear which specific executive functions need to be targeted. Hence, it will be of paramount importance to study systematically and comprehensively the different qualities of EFs in future studies of environmental causes for ADHD.

The literature suggests sex differences for the relative contribution of genes and environment on set shifting as measured by WCST, with a larger impact of genetic influences in female than male adolescents (Anokhin et al., 2010). While we did not find evidence for non-shared environmental influences on WCST-performance in boys, there was some evidence for environmental influences

on set-shifting and cognitive flexibility in girls.. From these results it could be hypothesized that impairments in planning and inhibitory control are more prominent features in boys than girls with ADHD traits.

The focus of the current study was to investigate if non-shared environmental factors impact on EFs in ADHD. Only one specific environmental factor; birth order, was explicitly examined in the dimensional part. In the categorically/clinically discordant pairs, a more thorough investigation of possible non-shared environmental factors was conducted. However, due to the limited sample size, these analyses should be viewed exploratory and inspiring hypotheses driven research. We have investigated birth weight, birth complications, viral and bacterial infections during the first years of life as potential non-shared environmental factors in the causes of ADHD. Within the ongoing RATSS study, a greater number of participants will enable more thorough analysis in the future, and these possible risk factors will be further addressed using for instance toxicological analyses of bio samples and epigenetic approaches. This discordant MZ twin study offers several methodological advantages. The design is powerful for detection of the involvement of non-shared environmental factors and it essentially eliminates possibly confounding effects. However, some identified environmental risk factors, such as parental socioeconomic status, maternal lifestyle factors, and psychosocial stressors during pregnancy are shared, and are therefore not investigated further in this study. On the other hand, shared environmental factors (environmental influences that make children growing up in the same family similar) are said to have a small or limited effect on ADHD (Barkley, 1998; Burt et al., 2012).

Another methodological advantage of the study is the statistical method applied, where the independent variable (ADHD traits) is treated as both continuous and categorical/clinical variable. Since the same result was replicated in both approaches, the findings are demonstrated to be fairly robust. *Limitations*

There are at least four limitations to the study that warrant consideration. One potential methodological draw-back is the reliance on one scale with a limited number of items for the assessment of ADHD traits. To address this limitation, stringent clinical assessments were made of all subjects included in the study. Analyses of pairs, qualitative discordant for clinical ADHD diagnoses, were analysed separately, with results showing a good agreement with the results from the dimensional analysis. In addition, the CBCL scale for AP is one of the most widely used scales in ADHD research, and is considered reliable with a moderate to high agreement with clinical ADHD diagnosis. The second limitation is the still relatively small sample size, especially for the categorical/clinical analyses of ADHD discordant pairs and sex differences. Therefore, replication in samples of larger numbers discordant for ADHD, as well as in larger samples of female pairs, are desirable. Large samples are particularly needed in the study of non-shared environment in ADHD etiology, as their assumed contribution is relatively small (less than 30%). Thereby there is an a priori reduced likelihood to detect small effects in small samples. Nevertheless, this is still the largest study of MZ twins discordant for ADHD traits to date, providing unique and novel data.

The third limitation is the fact that the twins were not taken off medication when participating in the study. Since a substantial number of the participants were medicated for ADHD symptoms at the time for assessment, true effects are likely to be diminished as those agents are known to improve cognitive functioning such as attention (Biederman et al., 2008). However, only three pairs were included in the study sample, in which only one and not both twins were medicated for ADHD symptoms, and analyses excluding these pairs did not alter the results. More likely, the intra-pairs difference would increase if the participants were taken off medication, and effects would be more prominent.

The last limitation is the circumscribed battery of neuropsychological tests used to measure executive functioning. Executive functioning is a complex set of functions, challenging to capture

in only two tests. Thus, future studies should more specifically address for instance inhibition (e.g. go/no-go tests), and working memory. *Conclusions*

In summary, three conclusions can be made from our main findings. First, we support the notion that inhibitory control is a cognitive marker of ADHD traits. This is in line with the literature and basically a replication of results found in several ADHD studies. Second, the results indicate that inhibitory control is associated with ADHD traits at least via non-shared environmental factors. Third, the findings support the quantitative continuum model of ADHD, since the findings hold for both quantitative discordant clinically enriched pairs as for clinical ADHD discordant pairs.

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Tables

TABLE 1. Demographic and clinical measures of monozygotic twin pairs discordant for ADHD traits (≥ 1 point intra-pair difference on the AP scale)

Clinical characteristics	Groups					
	Twin 1: Higher ADHD traits (n=27)			Twin 2: Lower ADHD traits (n=27)		
	Mean	SD	Range	Mean	SD	Range
Age	15.2	3.0	(11-20)	15.2	3.0	(11-20)
AP; PR	7.8	4.4	(1-15)	4.4	3.5	(0-13)
AP; SR	6.9	3.7	(0-14)	4.9	2.9	(0-11)
IQ (GAI)	89.8	17.7	(61-121)	92.9	17.4	(62-123)

Note: AP=Attention Problem, PR=Parent Report, SR= Self Report, GAI=General Ability Index; ADHD=Attention Deficits Hyperactivity Disorder; SD=Standard Deviation

TABLE 2. Descriptive results for intra-pair differences in male and female twin-pairs on the WCST and TT

Behavioral measures; intra-pair differences	Females (N=7 pairs)				Males (N=20 pairs)			
	Mean	SD	Range	Skew-ness	Mean	SD	Range	Skew-ness
AP; PR	2.57	0.98	(1-4)	-0.28	3.65	2.50	(1-11)	1.39
TT; Total achievement	2.57	4.7	(-4-9)	-.11	.05	4.13	(-6-9)	0.52
TT; Total Rule Violations	0.00	1.63	(-3-2)	-0.96	0.35	3.47	(-9-4)	-1.71
TT; Rule-Violation-Per-Item Ratio	0.01	0.18	(-0.3-0.2)	-0.80	-0.05	0.40	(-1.0-0.7)	-1.10
WCST; total errors	20.85	19.10	(0-56)	1.16	-2.65	17.62	(-46-28)	-0.76
WCST; perservative responses	5.00	4.00	(0-11)	-0.02	-1.56	10.89	(-27-17)	-0.67
WCST; perservative errors	32.29	18.63	(12-65)	0.94	13.4	12.41	(-6-37)	0.37

Note: AP=Attention Problem, PR= Parent Report, SD=Standard Deviation, TT=Tower Test; WCST=Wisconsin Card Sorting Test

TABLE 3. Correlations between intra-pair differences in ADHD traits (continuous variable) and intra-pair differences on measures of executive functioning in total sample (N=27 pairs)

Executive functioning measures	Correlation Coefficient	p value (1-tailed)
TT: Total achievement	-.023	.454
TT; Total Rule Violations*	.411*	.017*
TT; Rule-Violation-Per-Item Ratio*	.378*	.026*
WCST; Total Errors	.043	.415
WCST; Perservative Responses	.086	.335
WCST; Perservative Errors	.088	.332

Note: TT=Tower Test; WCST=Wisconsin Card Sorting Test

* p-value < .05

TABLE 4. Analyses of intra-pair differences in executive functioning in MZ pairs discordant for ADHD diagnoses measured as a categorical variable (N=4 pairs)

Executive functioning measures	Z value (Wilcoxon signed rank test)	p value (1-tailed)
TT: Total achievement	-1.461	.072
TT; Total Rule Violations	-1.633	.051
TT; Rule-Violation-Per-Item Ratio	-1.604	.054
WCST; total errors	1.095	.137
WCST; perservative responses	-0.730	.233

WCST; preservative errors -0.730 .233

Note: TT=Tower Test; WCST=Wisconsin Card Sorting Test

Captions to illustrations

FIGURE 1. Correlations between intra-pair differences on AP scale (CBCL/ABCL) and intra-pair differences on Tower, subscale total rule violation (D-KEFS). All values are standardized and presented as *Z* values.

Note: TT=Tower Test, AP=Attention Problem

Ethical approval

All parts of the project have been approved in full by the National Swedish Ethical Board, or responsible regional ethical review board (dnr 2010/1778-31/4; dnr Ö 32-2010, plus six amendments), and the Swedish Data Inspection Board.