The Role of Age in Association Analyses of ADHD and Related Neurocognitive Functioning: A Proof of Concept for Dopaminergic and Serotonergic Genes


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Elucidating genetic mechanisms involved in Attention-Deficit/Hyperactivity Disorder (ADHD) has been challenging. Relatively unexplored is the fact that genetic mechanisms can differ with age. The current study explored the association between dopaminergic and serotonergic genes, ADHD symptoms, and neurocognitive functioning in relation to age. Associations of three genetic ADHD risk factors, \textit{DAT1}, \textit{DRD4}, and \textit{5-HTT} with symptoms and six neurocognitive measures were explored in two samples of the NeuroIM AGE study: 756 children, adolescents, and young adults with ADHD, their siblings, and controls (M age 17 years, SD 3.2), and 393 parents with and without ADHD (M age 48 years, SD 4.8). Association analyses were performed in both samples, and effects were compared to address dichotomous age effects. Gene*age interactions were examined to address continuous age effects. Moderating effects of age were found for \textit{DRD4}-7R carriership and ADHD symptoms in the adult group only; in the adolescents the \textit{5-HTT} LL genotype was differentially associated with inhibition and with motor timing at different ages, and to inhibition in adults; \textit{DAT1} 10-6 haplotype carriership showed differential working memory performance depending on age. None of our effects survived correction for multiple comparisons. Our results are preliminary, but may point to differential genotype–phenotype associations at different ages. This can be seen as a proof of concept for the importance of age in dopaminergic and serotonergic genetic association analyses. Our findings are consistent with the idea that genetic and neurocognitive mechanisms underlying ADHD may change throughout life. © 2015 Wiley Periodicals, Inc.
INTRODUCTION

Attention Deficit/Hyperactivity Disorder (ADHD) is a highly prevalent neurodevelopmental disorder characterized by symptoms of inattention and/or hyperactivity and impulsivity [Diagnostic and Statistical Manual of Mental Disorders, DSM-5, 2013; Polanczyk et al., 2007]. ADHD often persists into adulthood [Turgay et al., 2012] and is substantially influenced by genetic factors [Thapar et al., 2013]. The heritability of ADHD is estimated to be ~76% [Faraone et al., 2005], but gene-finding in ADHD has been difficult. This is likely due to phenotypic and genetic heterogeneity in combination with very small effect sizes of common genetic variants contributing to ADHD risk [Gizer et al., 2009] and/or genetic risk factors of stronger effects being rare and non-specific [Poelmans et al., 2011; Thapar et al., 2013]. Associations with ADHD have been studied most extensively for candidate genes in the dopaminergic, serotonergic, and noradrenergic systems, all hypothesized to play a role in ADHD [Gizer et al., 2009; Banaschewski et al., 2010]. Dopaminergic genes are considered key candidates for ADHD because of the strong therapeutic effects of stimulant medication [Volkow et al., 2002; Faraone and Buitelaar, 2010; Del Campo et al., 2011]. A central role of serotonergic genes is suggested by the effects on (early) brain development [Azmitia, 2001], the influence on behavioral traits like impulsivity [Banaschewski et al., 2010; Landas et al., 2010], and the interaction with dopamine [Oades, 2008]. Noradrenergic genes have also received considerable interest in ADHD research [Banaschewski et al., 2010], but a role for these genes could not be confirmed in meta-analysis [Gizer et al., 2009]. To date, the dopamine transporter gene DAT1/SLC6A3, the dopamine receptor 4 gene DRD4, as well as the serotonin transporter gene 5-HTT/SLC6A4 have been most extensively studied in ADHD. These three genes have repeatedly been associated with ADHD, although with substantial heterogeneity across—and between—childhood and adult studies [Gizer et al., 2009; Li et al., 2006; Franke et al., 2012]. This suggests the need to include possible moderating factors of these associations in future studies.

A potential explanation for the inconsistencies observed in association studies is the effect of age. A developmental approach has been used scarcely when studying the relationship between genes, the ADHD phenotype, and related neurocognitive dysfunction. Longitudinal designs suggest that in particular hyperactive/impulsive ADHD symptoms decrease with age [Biederman et al., 2000; Larsson et al., 2006]. Given that expression levels of genes can differ across different stages of development [Elia and Devoto, 2007], the contribution of risk genes to ADHD may not be constant across the life course. Support for the latter theory has come from studies applying a longitudinal twin design [Larsson et al., 2004; Greven et al., 2011]. A specific well-known example is the association of a different DAT1 haplotype with ADHD in adults (the 9-6 haplotype) compared to the haplotype associated in children (the 10-6 haplotype) [Franke et al., 2008, 2010]. In addition, previous (twin) studies have shown that genetic influences on neurocognitive ability increase with age [Boomsma et al., 2002; Plomin and Spinath, 2004; Polderman et al., 2007]. These results imply that age is an important factor to take into account in molecular genetics analyses in ADHD.

A genetic design could further benefit from the inclusion of well-studied ADHD-related neurocognitive (dys) functions, since these ‘endophenotypes’ have been hypothesized to have a less complex genetic architecture than the disease phenotype, and they can provide further insight into the cognitive or neural mechanism that underlies ADHD [Gottesman and Gould, 2003; Croce et al., 2008, 2013]. Although the causal role of neurocognitive deficits in ADHD remains uncertain [Coghill et al., 2014], results from several genetic studies support the utility of neurocognitive measures by showing an association of ADHD-related cognitive impairments with ADHD-related dopaminergic and serotonergic genes. Still, the exact influences of specific alleles of these candidate genes in ADHD-related neurocognitive functioning remain unclear as some conflicting results have been found for both DAT1 (10R allele of the 3’UTR (untranslated region) VNTR polymorphism) (see for review [Rommelse et al., 2009b] and DRD4 (7R allele of the exon 3 VNTR polymorphism) (see for reviews, Kebir et al., 2009; Wu et al., 2012). For 5-HTT (S and L allele of the 5-HTTLPR variant) (cognitive studies including ADHD patients and/or healthy subjects are emerging and show conflicting results as well [Oades, 2007, 2008; Paaver et al., 2007; Lane et al., 2008; Anderson et al., 2012; Zilles et al., 2012]. As neurocognitive impairments in ADHD have been found to vary with age [Seidman, 2006; Thissen et al., 2014] part of the conflicting results might be attributable to the different age ranges investigated in different studies.

The main aim of the current study was to explore the influence of age on the relationship of several well-known ADHD candidate genes with disorder symptoms and ADHD-related neurocognitive deficits. Age was investigated by including an offspring sample with a broad age range (from late childhood, throughout adolescence, until young adulthood) and a sample of their middle-aged adult parents. Since previous ADHD genetic studies mainly focused on children and young adults, the inclusion of adolescents and middle-aged adults might provide novel insights with regard to the influence of age. Further, by including two generations from the same gene-pool (i.e., parents and offspring), the effect of different ages between the samples can be compared while minimizing the effects of population differences. Candidate genes—DAT1, DRD4, and 5-HTT—were selected based on the aforementioned evidence of association with ADHD (e.g., [Gizer et al., 2009]). The selection of neurocognitive measures—working memory, motor inhibition, timing abilities, and reaction time variability—was based on their robust association with deficits in children with ADHD ([working memory]; see for meta-analyses [Martinussen et al., 2005; Kasper et al., 2012], [motor inhibition]; see for review [Willcutt et al., 2005] [timing abilities]; [Toplak et al., 2006; Noreika et al., 2013], [reaction time variability]; [Castellanos and Tannock, 2002; Klein et al., 2006; Frazier-Wood et al., 2012] and their unaffected siblings [Kuntsi et al., 2013; Rommelse et al., 2008a; Rommelse, 2008; Rommelse et al., 2008c], and based on previously reported associations with our genes of interest ([DRD4]; Altink et al. [2012] [5-HTT];[Anderson et al., 2012]; e.g., [DAT1];Bellgrove et al., 2005; Durston et al., 2005; Froehlich et al., 2007; Paaver et al., 2007; Soderqvist et al., 2012; Zilles et al., 2012].

MATERIALS AND METHODS

Participants

All participants (parents and offspring) were selected from the Dutch follow-up (2009–2012) of the International Multicenter ADHD Genetics (IMAGE) study performed between 2003 and
2006 (as described previously in Nijmeijer et al. [2009]). Inclusion criteria for offspring at IMAGE enrolment were; age between 5 years and 19 years, European Caucasian descent, IQ > 70, and no diagnosis of autism, epilepsy, general learning difficulties, brain disorders or known genetic disorders (such as Fragile X syndrome or Down syndrome). All families were re-invited to a follow-up assessment, with a mean follow-up period of 5.9 years (SD = 0.72) and no additional inclusion criteria, except a minimum age of 8 years for neurocognitive testing and MR imaging. Diagnostic status and ADHD symptom severity were re-assessed at follow-up. The current study involved 255 ADHD families and 88 healthy control families with at least one family member with available genotype data and complete neurocognitive assessment. The ADHD families consisted of 396 ADHD cases, 188 unaffected siblings, and 393 parents (156 affected, 237 unaffected). The control families consisted of 172 offspring family members. In the offspring sample (hereafter referred to as adolescents) >90% was 12 years of age, and only biological offspring (in both ADHD and control families) were included. The offspring sample consisted of children, adolescents and young adults (age range 8–25) with >92% being 12 years or older and >84% being 20 years or younger. The parent sample included biological parents from ADHD families only. Table I provides participants’ characteristics.

### ADHD Diagnoses

All participants were assessed using a semi-structured diagnostic interview (Dutch translation of the Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime Version (K-SADS-PL) [Kaufman et al., 1997] and Conners’ ADHD questionnaires to determine current ADHD diagnosis. A diagnostic algorithm was applied to combine symptom counts on the K-SADS-PL and Conners’ questionnaire in both samples. A detailed description of the algorithm is provided in the Supplementary materials Methods S1.

### Measures

**ADHD symptoms.** Information concerning current ADHD symptoms was obtained using the Dutch version of the Conners Adult ADHD Rating Scales—Observer: Screen Version (CAARS-O:SV) for adults and the Conners Parent Rating Scales—Revised Long version CPRS-R:L for children and adolescents [Conners et al., 1999]. For each adult, scores on the ADHD Inattentive and Hyperactive-Impulsive scales of CAARS were used as separate measures of current adult ADHD severity. This allowed examination of possible differential effects for inattention and hyperactivity-impulsivity. The same method was used for children and adolescents, using the CPRS subscales ADHD Inattentive and Hyperactive-Impulsive.

**Neurocognitive tasks.** Table II provides a brief description of the six neurocognitive variables used for association analyses. Details on each paradigm are provided elsewhere [Leth-Steensen et al., 2000; Rommelse et al., 2008a; Thissen et al., 2014]. All measures have shown an association with ADHD in this sample [Thissen et al., 2014a; Thissen et al., 2014b].

### Procedure

This study was part of a comprehensive assessment protocol (see for an overview von Rhein et al., 2014) also containing functional and structural Magnetic Resonance Imaging (MRI). MRI scans were not recorded in parents. Participants were asked not to use psychostimulant drugs from 48 hr before the assessment. For
other psychotropic medication participants were asked not to use it at least 3 days before the assessment. After the study procedures had been carefully explained, all participants gave their written informed consent, with parents providing consent for children <12 years of age. The study protocol was approved by the local medical ethics committees of Radboud University Medical Center in Nijmegen, and the VU Medical Center in Amsterdam.

Genotyping

An extensive description of DNA extraction and genotyping in IMAGE is provided elsewhere [Brookes et al., 2006]. Briefly, for the IMAGE sample DNA was extracted from blood samples or immortalized cell lines at Rutgers University Cell and DNA Repository, New Jersey, USA. Variable number of tandem repeat (VNTR) polymorphisms from exon 3 of the DRD4 gene, the 3′-untranslated region (UTR) and intron 8 of the DAT1/SLC6A3 gene, and the promoter region of the 5-HTT/SLC6A4 gene (HTTLPR) had been genotyped for previous studies by the IMAGE consortium [Brookes et al., 2006; Xu et al., 2008]. Standard PCR protocols were used for all VNTR markers and amplified products were visualized on 2% agarose under UV light. Extra samples from the NeuroIMAGE sample which had not undergone genotyping in IMAGE were genotyped at the Department of Human Genetics of the Radboud University Medical Center. DNA for those samples was isolated from saliva using Oragene containers (DNA Genotek, Ottawa, Ontario, Canada) according to the protocol supplied by the manufacturer. VNTRs were genotyped using standard PCR protocols. After the PCR, fragment length analysis was performed on the ABI Prism 3730 Genetic Analyser (Applied Biosystems, Nieuwekerk a/d IJssel, The Netherlands) and results were analyzed with GeneMapper® Software, version 4.0 (Applied Biosystems). No deviations from Hardy Weinberg equilibrium were found for the VNTR polymorphisms (DRD4 exon 3: $P = 0.15$, DAT1 3′-UTR: $P = 0.78$, DAT1 intron 8: $P = 0.55$, HTTLPR: $P = 0.13$). From the genotype of the DAT1 3′-UTR and intron 8 VNTRs, a haplotype was calculated using the HaPloStats package (R version 2.12.0) [Schaid et al., 2002] (see Table III).

Data Analyses

ADHD measures and cognitive measures were normalized and standardized into z-scores (Van der Waerden transformation) for adults and adolescents separately. For stop signal reaction time (SSRT) of the Stop task, motor timing variability and tau, z-scores were mirrored, so that higher z-scores for all measures would have the same direction: better performance or more severe ADHD symptomatology. Reaction time median (RTM) was an exception, because a higher or lower median reflected a better or worse performance depending on how close this median was to the 1 s interval that needed to be reproduced. Performance ceiling effects did not occur on any of the tasks, as indicated by boxplot analyses with raw data in both samples (not shown).

Generalized estimating equations (GEE) were used with a linear regression model and robust estimators. To correct for familial dependence within the data set, family number was used as repeated measure and the structure for working correlation matrices was set at exchangeable. Only participants with available genotyping and

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### TABLE II. Description of the Neurocognitive Tasks and Variables

<table>
<thead>
<tr>
<th>Task</th>
<th>Measurement potential(s)</th>
<th>Dependent variable(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor Timing Task (1 s interval)</td>
<td>Timing accuracy</td>
<td>Median reaction times (in ms)</td>
</tr>
<tr>
<td></td>
<td>Reaction time variability</td>
<td>Ex-Gaussian component τ</td>
</tr>
<tr>
<td>Stop Task</td>
<td>Infrequent slow response times</td>
<td>Stop Signal Reaction Time (SSRT)</td>
</tr>
<tr>
<td>Visuospatial Grid Task</td>
<td>Speed of inhibition</td>
<td>Number of correct trials</td>
</tr>
<tr>
<td>Digit Span (WISC/WAIS-III)</td>
<td>Visuospatial working memory</td>
<td>Digit span backwards</td>
</tr>
<tr>
<td>Vocabulary and Block Design (WISC/WAIS-III)</td>
<td>Verbal working memory</td>
<td>Total IQ-score</td>
</tr>
</tbody>
</table>

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### TABLE III. Genotypes for the Genetic Variants in the Candidate Genes

<table>
<thead>
<tr>
<th>DRD4 (7R-carriers/non-carriers)*</th>
<th>Adults</th>
<th>Children/adolescents/young adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>77/148</td>
<td>243/422</td>
<td></td>
</tr>
<tr>
<td>37/114/66</td>
<td>95/330/236</td>
<td></td>
</tr>
<tr>
<td>55/328</td>
<td>681/58</td>
<td></td>
</tr>
</tbody>
</table>

*For complete genotyping information see Supplementary Table 1

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Details on each of the paradigms are provided elsewhere [Leth-Steensen et al., 2000, Rommelse et al., 2008, Thissen et al., 2014]. Adolescents performed these tasks during fMRI-measurement. Wechsler Intelligence scale for Children or Wechsler Adult Intelligence Scale.
neurocognitive data were included in the analysis (Table III). Combining adolescents and parents in one sample produced a non-normal age distribution, therefore effects were analyzed in the adolescent and adult sample separately. We first tested whether the VNTR variants of \textit{DRD4} (presence or absence 7R risk allele), \textit{DAT1} (presence or absence of at least one 10-6 risk haplotype in adolescents; presence or absence of at least one 9-6 risk haplotype in adults), or \textit{5-HTT} (presence of two [LL], one [SL], or no [SS] risk alleles) predicted ADHD diagnosis, inattentive and hyperactive/impulsive ADHD symptoms, and cognitive functioning. For prediction of diagnosis, odds ratios were calculated, including two allele groups for \textit{5-HTT} (LL vs. other, and SS vs. other). Effects of age, gender, and the interaction of risk allele or haplotype with age were included and kept in the model if nominally significant. Two levels were applied for the main gene effects on ADHD symptoms (two symptom domains), and six levels were applied for the main gene effects on neurocognitive functioning (six measures). Because the obtained symptom counts and neuropsychological measures are correlated, correction for multiple comparisons was undertaken by calculating the effective number of independent tests \((\text{Meff})\) based on the observed eigenvalue variance using the matSpD interface (http://genepi.qimr.edu.au/general/daleN/matSpD; [Nyholt, 2004; Li and Ji, 2005]). This analysis showed that the original eight tests corresponded to seven independent tests. To correct for multiple testing and to determine the significance of the results Bonferroni correction was applied by dividing the significance level by the number of independent tests. As three genetic variants were tested, as well as two levels (adults and adolescents), a \(P\)-value of 0.05/7/3/2 = 0.00119 was considered significant. Effects were described as ‘nominal’ in case significant at the 0.05 level, but not surviving multiple comparison correction. Post-hoc we investigated if results were altered if IQ was included as a covariate, as the inclusion of IQ in ADHD research is an active debate [Rommelse et al., 2008c].

If none of the genes was potentially related to a dependent measure (ADHD symptoms or cognitive functioning) in the adolescent or adult sample, we investigated whether the effects for adolescents or adults were significantly different by defining and testing contrasts for the regression weights associated with the relations.

Missing data were less than 1.5% (adolescents) and 2% (adults) for cognitive measures, except for adolescent infection (48% missing) and visuospatial working memory (46% missing). Visuospatial working memory and inhibition in adolescents were measured during fMRI. Measurement was performed in a random subgroup (75% of sample) because of time constraints in the assessment protocol, and MRI was contraindicated in about 25% of the children (e.g., because of wearing braces). Missing data were not imputed. For analyses including \textit{DRD4}, \textit{DAT1}, and \textit{5-HTT} in the adolescent sample, \(n = 665\), \(n = 739\), and \(n = 661\) subjects were available, respectively. For analyses in the adult sample including \textit{DRD4}, \textit{DAT1}, and \textit{5-HTT}, \(n = 225\), \(n = 386\), and \(n = 217\) subjects were available, respectively.

**RESULTS**

**Genetic Association With ADHD Diagnosis and Symptoms**

The results of the association between clinical diagnosis of ADHD and \textit{DRD4}-7R, the \textit{HTTLPR}, and the haplotype in \textit{DAT1} are provided in Table IV. In adults, we found nominally significant association between ADHD and \textit{DRD4}-7R (Odds ratio: 0.50, \(P = 0.02\)): 25% of adults with ADHD were 7R carriers, whereas 40% of adults without ADHD were 7R carriers, suggesting a possible protective effect of the 7R allele against adult ADHD. In line with this, a nominal effect of \textit{DRD4}-7R was found on ADHD symptoms, both inattentive and hyperactive/impulsive, in the adult group. Carriers of the 7R allele had less severe symptoms (Cohen’s \(d = −0.39\) [\(P = 0.027\)] and −0.42 [\(P = 0.018\)], respectively). No association between \textit{7R} and ADHD diagnosis or symptoms was found in the adolescent sample. Only the effects between \textit{DRD4} and inattentive symptoms differed from the adults (\textit{contrast} \(P = 0.045\)).

**Genetic Associations with Neurocognitive Functioning**

**\textit{DRD4}**. None of the associations between neurocognitive functioning and \textit{DRD4} survived multiple testing correction.

**\textit{5-HTT}**. No main effects were found for the \textit{HTTLPR} on neurocognitive functioning. However, two nominal interaction effects of \textit{HTTLPR}×\textit{age} were found. The first was found for inhibition in adolescents (\(P = 0.037\)). Until 17 years of age (age cut-offs based on fitted regression line), LL homozygotes performed better than SS homozygotes on inhibition (Cohen’s \(d = 0.47\) \(P = 0.029\)). This effect vanished at the age of 17 years. The \textit{HTTLPR}×\textit{age} effect on inhibition in adolescents did not differ from the \textit{HTTLPR}×\textit{age} effect on inhibition in adults (\(P = 0.009\)) (before age 51 years, the LL genotype performed better than the SS

| TABLE IV. Association Between Clinical Diagnosis of ADHD and Genetic Variants |
|-------------------------------------------------|-----------------|-----------------|
|                    | **Children/adolescents/young adults** | **Adults** |
| \textit{DRD4}-7R OR [p-value]                      | 0.80 [0.24]  | 0.50 [0.02] |
| \textit{HTTLPR} [LL vs other] OR [p-value]           | 0.74 [0.13]  | 0.76 [0.37] |
| \textit{HTTLPR} [SS vs other] OR [p-value]           | 0.76 [0.27]  | 1.00 [0.99] |
| \textit{DAT1} 10R-6R OR [p-value]                   | 0.76 [0.60]  | —               |
| \textit{DAT1} 9R-6R OR [p-value]                    | —               | 1.10 [0.76] |

Note Odds Ratio (OR) and \(p\)-values of the \(\chi^2\) association with ADHD diagnosis.
genotype \(P = 0.02\), Cohen’s \(d = 0.55\); after age 50 years, the SS genotype performed better than the LL genotype \(P = 0.025\), Cohen’s \(d = 0.71\) \((\text{contrast} \ P = 0.53)\).

The HTTLPR*age interaction was also nominally significant for motor timing in the adolescents \(P = 0.008\). Until age 19 years SS genotype was associated with better motor timing and LL genotype with worse motor timing \(P = 0.024\) Cohen’s \(d = 0.31\), while after age 19 the LL genotype was associated with better motor timing \(P = 0.034\) Cohen’s \(d = 0.42\). The HTTLPR*age effect was non-significant in the adults.

**DAT1.** None of the associations between neurocognitive functioning and DAT1 survived multiple testing correction. A nominally significant interaction effect of DAT1*age was found on verbal WM in adolescents \(P = 0.025\) \((\text{between age} 14\) years and 18 years, 10-6 non-carriers performed better than carriers of this haplotype \([\text{Cohen’s} \ d = 0.48, \ P < 0.001]\); after age 18 years, 10-6 carriers did not perform differently from non-carriers \([\text{Cohen’s} \ d = 0.50 \ P = 0.12]\). A summary of all reported associations can be found in supplementary Table 2.

**IQ.** Post-hoc analyses including IQ as a covariate did not significantly change our results (results not shown).

**DISCUSSION**

The current study contributes to ADHD research by exploring the influence of age on the relationship of three well-known ADHD candidate genes with ADHD symptoms and ADHD-related neurocognitive functions. Results suggest potential differential involvement of genetic factors in ADHD at different stages of life. The dopamine receptor DRD4-7R allele was nominally associated with less ADHD inattentive symptoms in adults selectively, which differed significantly from the association in the adolescent sample. The serotonin transporter HTTLPR LL genotype was nominally associated with better inhibition in adolescents, until the age of 17 years; in the adult group the LL genotype was also nominally associated with better inhibition, which changed to an association with worse inhibition after age 50 years. For motor timing a nominal interaction effect between age and HTTLPR was found with the LL genotype performing worse before the age of 19 and better after age 19 years. A nominal interaction effects between DAT1 and age on verbal working memory in adolescents was also found, with the childhood risk haplotype (10-6) carriers performing worse before the age of 18 years.

The nominal association between DRD4-7R and ADHD symptom severity in adults appears counterintuitive, as the 7R allele has generally been reported as the risk allele for ADHD [Li et al., 2006]. However, previous results are inconsistent, with some reporting the opposite or no effect of the 7R allele, with most reports investigating childhood ADHD [Gizer et al., 2009]. As dopamine transporter density decreases with age [Spencer et al., 2005], and ADHD symptoms decrease during adolescence [Biederman et al., 2000], the protective effect of the 7R allele during adulthood might reflect changes in the dopaminergic system. Indeed, longitudinal analysis of associations between the DRD4-7R allele and (ADHD-related) cortical thickness showed association in early development, but this association was no longer found around age 16–18 years [Shaw et al., 2006]. Clinically, a better outcome for adolescent 7R carriers has been reported [Shaw et al., 2007]. In contrast, in adult samples, the 7R allele has been associated with increased levels of ADHD and worse performance on neurocognitive tasks [Muglia et al., 2000; Congdon et al., 2008], although associations to better cognitive performance have been reported as well [Boonstra et al., 2008]. The studies by Congdon and others and Muglia and others included younger adult samples (mean age 20 and 34 years, respectively) than our study and the study by Boonstra and others (mean age 39 years), which supports the idea that throughout adulthood the effects of dopamine genes on ADHD-related measures are not static. This is in line with results showing a trend towards protective effects of the 7R allele in older adults with ADHD in a sample with an age range up to ~65 years [Johansson et al., 2008]. In general, it might be tentatively suggested that the 7R allele may have a protective effect in adult ADHD, only emerging during or after middle-late adulthood, but not yet (consistently found) during adolescence and young adulthood.

Our age-dependent findings of HTTLPR on both inhibition and motor-timing, showing opposite effects, may point to a pleiotropic effect of this gene. The effects may suggest that optimal serotonin levels (high or low) differ with age, which at these specific developmental stages might be related to increased risk for expressing ADHD-related behaviors like motor timing, though results are in the opposite direction for inhibition. Inconsistent results have been reported for HTTLPR in ADHD, however, most of the studies in childhood ADHD report the L allele as the risk allele [Beitchman et al., 2003]. A large meta-analysis [Landaas et al., 2010], which examined the association of the 5-HTT gene with adult ADHD, did not find the L allele, but rather reported a slight overrepresentation of the S allele in adult ADHD, although this did not reach significance. This reported trend is in line with our results for motor timing, but opposite to our inhibition finding, and might indicate differential involvement of serotonin genes in young and middle adulthood compared to childhood in ADHD-related neuropsychological traits. However, the adult group showed another switch at age 50, where after the age of 50 the SS genotype performed better than the LL genotype. The directionual outcome of altering serotonin transporter levels may thus depend on developmental stage, as also suggested by others [Wiggins et al., 2014]. As the S allele is related to lower transcription activity compared to the L allele [Lesch et al., 1996], our results might reveal a switch in the influence of serotonin with the optimal levels differing with age. That is, in our sample including subjects with and without ADHD, before young adulthood, lower serotonin transcription activity (S allele) seems beneficial for motor timing but detrimental for inhibition, whereas during (and possibly after) young adulthood an increase (L allele) leads to better motor timing, but worse inhibition, with a switch for inhibition around age 50 where low serotonin becomes beneficial for inhibition again.

The nominal interaction between age and DAT1 10-6-carriership on verbal WM, which switched at age 18 years, is in line with previous results for clinical ADHD phenotypes [Franke et al., 2008; Franke et al., 2010]. However, more research is needed to unravel the (age-related) underlying mechanisms of DAT1 in ADHD-related cognitive functioning.

Our findings should be viewed in light of certain strengths and limitations. We were able to examine a rather large, well-phenotyped sample of participants with ADHD and healthy controls, although our sample size is modest given the small effect sizes of most genetic
factors. Through the availability of adolescents and middle-aged adults—not yet structurally investigated in molecular genetic research on ADHD before—this study helps to fill a gap in the current literature. We improved upon previous studies by minimizing population differences because the adult and child/adolescent/young adults sample included parents and their offspring, which made both age groups well comparable. Moreover, because both groups were assessed as part of the same study, methodological differences used to assess both groups were minimized. As age effects were derived from a cross-sectional design, however, they should be considered preliminary until replication from longitudinal designs. Additionally, reported age effects were only nominally significant and therefore should be considered as exploratory and in need of replication. Further, we were not able to replicate associations between a clinical diagnosis and \( DAT1 \) and \( 5\text{-}HTT \), although, as part of a large international study using childhood clinical diagnoses assessed earlier, the current sample did contribute to a significant association between \( DAT1 \) haplotype and diagnosis during childhood [Brookes et al., 2006]. Potential explanations for not finding an association between \( DAT1 \) and ADHD diagnosis in the current study is the follow-up nature of our sample, where participants were re-diagnosed. Additional analyses investigating gene–gene interactions were not followed due to the moderate sample size of the current sample. However, they can be of importance for follow-up studies as genetic interactions have been described for ADHD previously [Carrasco et al., 2006; Gabriela et al., 2009].

In sum, we found differential genotype–phenotype associations in different age groups: effects for \( DRD4 \) were selective for adults, while effects for \( 5\text{-}HTT \) were found in adolescents (inhibition and motor timing) and adults (inhibition). Our results are exploratory, did not survive multiple testing, and are in need of replication. Nonetheless, they can be considered as a proof of concept for the importance of age as they suggest age to be a key factor in genetic association analyses of ADHD and related neurocognitive functioning. Neurocognitive measures might be more sensitive to the effects of genetic variation than clinical symptoms.

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