Neuropsychological Measures Probably Facilitate Heritability Research of ADHD

Submitted as:
Chapter 11

Abstract

Previous studies, in which cognitive and motor neuropsychological tasks were administered to 816 children from ADHD- and control-families, showed that various of these measures appeared useful for genetic research in ADHD by forming candidate endophenotypes: underlying, heritable, vulnerability traits that mark an enhanced liability for developing ADHD. The current study extends these findings by showing that six of these ten measures correlate more strongly between siblings than an ADHD composite, suggesting these measures may have a larger heritability than ADHD itself. Significant sibling cross-correlations also suggested that six of ten neuropsychological measures related to similar familial (and heritable) factors as ADHD, suggesting these measures to be useful for genetic research in ADHD. An aggregated neuropsychological composite appeared to be the most powerful, since it correlated more strongly between siblings than most individual task measures. These findings suggest heritability research in ADHD will probably be facilitated by including neuropsychological measures.
Chapter 11

**Introduction**

Neuropsychological research may aid in the discovery of genes and their influence on neuropsychiatric disorders. It has been hypothesized that neuropsychological deficits may form more direct expressions of disease genes than phenotypic symptoms (Castellanos & Tannock, 2002; Doyle et al., 2005c; Rommelse et al., 2007a; Waldman, 2005). In this framework, neuropsychological deficits act as ‘endophenotypes’; underlying, heritable, vulnerability traits that mark an enhanced liability for developing a disorder (Almasy & Blangero, 2001). Using endophenotypes can facilitate gene detection by forming more homogeneous subgroups of patients all being impaired in a specific endophenotype and/or by linking specific gene variations to endophenotypes.

Not all neuropsychological deficits are automatically suitable as endophenotypes. Various criteria have been postulated to define an endophenotype. The most frequently cited ones are: the neuropsychological deficit (1) should be associated with the disorder, (2) should be present in non-affected relatives to a higher degree compared to controls, (3) should correlate between biological family-members, and (4) should (partly) arise from the same heritable factors that also influence the phenotype (Almasy & Blangero, 2001; Castellanos & Tannock, 2002; Doyle et al., 2005b; Gottesman & Gould, 2003; Rommelse et al., 2007a; Skuse, 2001; Waldman, 2005). In order to examine whether certain neuropsychological deficits may be useful as endophenotypes, it is necessary to include both affected individuals and their non-affected relatives.

The current study deals specifically with candidate neuropsychological endophenotypes of Attention-Deficit/Hyperactivity Disorder (ADHD; American Psychiatric Association, 1994). Numerous studies have been conducted in the past decades, which have shown that a wide variety of neuropsychological deficits is associated with ADHD. Deficits have been observed in executive functions such as inhibition, working memory, set shifting and planning (Barkley, 1997; Boonstra et al., 2005b; Clark et al., 2000; Doyle, 2006; Pennington & Ozonoff, 1996; Seidman et al., 2004; Sergeant et al., 2002; Willcutt et al., 2005a), attentional skills (Barkley, 1997; Brodeur & Pond, 2001; Losier et al., 1996), time (re)production and estimation (Barkley et al., 2001a; Kerns et al., 2001; Toplak et al., 2006), (oculo)motor speed, variability, timing, and coordination (Leth-Steen sen et al., 2000; Pitcher et al., 2002; Rubia et al., 2003; Sergeant et al., 2006; Toplak et al., 2006; Van der Stigchel et al., 2007b; Van Meel et al., 2005), and sensori-motor integration (Yordanova et al., 2001). However, since relatively few studies have included non-affected relatives of patients with ADHD, it is unclear whether these
neuropsychological deficits may be useful as endophenotypes. The available studies that have included non-affected relatives targeted mainly executive functions as candidate endophenotypes. Two studies found no conclusive evidence of executive dysfunctioning in non-affected siblings (Nigg et al., 2004; Seidman et al., 2000) but another study did (Bidwell et al., 2007). Other studies specifically targeting inhibition or interference control, found evidence for these executive functions as endophenotypes (Crobie & Schachar, 2001; Doyle et al., 2005c; Schachar et al., 2005; Slaats-Willemse et al., 2003; Slaats-Willemse et al., 2005a). Less attention has been given to studying functions outside the executive domain. Previous studies have shown that non-affected siblings have (subtle) problems, similar to their affected siblings, in stability of motor control (Slaats-Willemse et al., 2005b), in motor/response variability (Andreou et al., 2007; Bidwell et al., 2007), and speed of oculomotor control (Van der Stigchel et al., 2007b). In five recent studies including 816 children (aged 5-19 years) from ADHD- and control-families (Rommelse et al., 2007a, b, c, d, 2008a), we found support for neuropsychological endophenotypes in the cognitive (inhibition, visuo-spatial and verbal working memory) and motor domains (motor speed, variability, timing and control): non-affected siblings of children with ADHD showed subtle deficits comparable to their affected siblings and siblings resembled each other on the neuropsychological tasks. Three issues regarding the utility of these neuropsychological measures as endophenotypes remained unanswered and are the aims of the current study.

First, it is expected that candidate neuropsychological endophenotypes are more strongly linked to heritable factors than the ADHD phenotype itself. If so, sibling correlations should be higher for the neuropsychological measures than for the ADHD symptoms. Second, it is expected that neuropsychological measures form a link between disease genes and the ADHD phenotype (Kuntsi & Stevenson, 2001). In that case, the neuropsychological measures will relate to the same familial (and with that, heritable) factors as the ADHD phenotype. Third, it is expected that the most powerful neuropsychological instrument is a composition score of individual neuropsychological measures, since a composition score entails less error variance than individual measures. If so, this composite will show better results in the analyses of the first and second research aim than the individual task measures. These three issues will be investigated in the current study.
Chapter 11

Methods

Participants

Families with at least one child with the combined subtype of ADHD (proband) and at least one additional sibling (regardless of possible ADHD-status) were recruited in order to participate in the Dutch part of the International Multicenter ADHD Genes study (IMAGE). The IMAGE project is an international collaborative study that aims to identify genes that increase the risk for ADHD using QTL linkage and association strategies (Brookes et al., 2006). Additional control families were recruited from primary and high schools from the same geographical regions as the participating ADHD-families. Controls and their first degree relatives were required to have no formal or suspected ADHD diagnosis. A total of 238 ADHD-families and 147 control-families fulfilled inclusion and exclusion criteria. Within the ADHD-families, 238 probands (all with combined subtype ADHD), 112 affected siblings (64 with combined subtype, 28 with inattentive subtype and 20 with hyperactive-impulsive subtype) and 195 non-affected siblings participated. Control-families consisted of 271 children. For 51 control children, no additional control sibling could be recruited for the study, because the sibling was unwilling to participate or because the control-family consisted of only one child. This sample allowed us to calculate a total of 540 sibling correlations.

All children were between the ages of 5 and 19 years and were of European Caucasian descent. Participants were excluded, if they had an IQ < 70, a diagnosis of autism, epilepsy, general learning difficulties, brain disorders or known genetic disorders, such as Down syndrome or Fragile-X-syndrome.

Both the proband and his/her siblings were similarly screened using the standard procedures of the IMAGE project described elsewhere (Brookes et al., 2006; Rommelse et al., 2007a). Briefly, screening questionnaires (parent and teacher Conners’ long version rating scales [Conners, 1996] and parent and teacher Strengths and Difficulties Questionnaires [Goodman, 1997]) were used to identify children with ADHD symptoms. T-scores ≥ 63 on the Conners’ ADHD-subscales (DSM-IV Inattention, DSM-IV Hyperactive-Impulsive, and DSM-IV Total) and scores > 90th percentile on the SDQ-hyperactivity scale were considered clinical. A semi-structured, standardized, investigator-based interview was administered for the child with ADHD: The Parental Account of Children’s Symptoms (PACS; Taylor, 1986). For details of the standardized algorithm that was applied to derive each of the 18 DSM-IV ADHD symptoms, readers are referred to Rommelse et al. (2007a). The Conners’ long version for both parents and
Chapter 11

teachers was completed for control children. Control children had to obtain non-clinical scores on both the parent and teacher version (Conners DSM-IV Total; T-score ≤ 62).

Full-scale IQ was estimated by four subtests of the WISC-III or WAIS-III (depending on the child’s age): Vocabulary, Similarities, Block Design and Picture Completion (Wechsler, 2000, 2002). These subtests are known to correlate between .90-.95 with the Full-scale IQ (Groth-Marnat, 1997). IQ testing took place while the children were off medication.

Experimental Tasks

The ten experimental tasks described in this study have been fully described elsewhere (Rommelse et al., 2007a, b, c, d, 2008a). A short description of each task will be given below. Based on previous results (Rommelse et al., 2007a, b, c, d, 2008a), the variable per task that showed most optimal results in the endophenotypic analyses was chosen for the current analyses.

Cognitive Tasks

Stop Task

The Stop Task was used to measure speed and accuracy of inhibition of an ongoing response (Logan, 1994; Logan & Cowan, 1984). Subjects were presented two types of trials: go-trials and stop-trials. Go-trials consisted of the presentation of a go-stimulus (drawing of a plane) that was either pointing to the right or to the left (Scheres et al., 2006). Children were instructed to press a response button that corresponded to the direction of the stimulus as quickly and as accurately as possible. Stop-trials were identical to the go-stimulus but in addition a stop-signal was presented (drawing of a cross that was superimposed on the plane). Children were required to withhold their response to the stop-signal. Go stimuli were displayed for 1000 ms, preceded by a 500 ms fixation point. Stop signals were displayed for 1000 ms minus delay time. Inter-trial intervals were 3000 ms. The delay between the go- and stop-signal was dynamically varied so that it could be estimated when the child successfully inhibited 50% of the stop-trials, and unsuccessfully inhibited the other 50%. At this point, the go-process and stop-process were of equal duration, which made it possible to estimate the latency of the stop-process: the Stop Signal Reaction Time (SSRT) (Logan, 1994). A total of 2 practice blocks and 4 experimental blocks were administered, each consisting of 60 trials. The first practice block consisted of only go-trials. The second practice block and the 4 experimental blocks consisted of 75% go-trials and 25% stop-trials. Go- and stop-trials were pseudo-randomly presented. Task administration took about 15 minutes. Based on previous results, the dependent measure was the SSRT
(Rommelse et al., 2008a), which showed endophenotypic-like group differences and correlated between siblings.

**Shifting Attentional Set**

Shifting Attentional Set was designed to measure speed and accuracy of motor inhibition and cognitive flexibility (De Sonneville, 1999). The task consisted of three blocks of which the first block was designed to acquire a baseline of the speed and accuracy of responding with which the performance on the second (motor inhibition) and third (cognitive flexibility) block could be compared. In all blocks, trials consisted of a horizontal bar with ten grey squares presented permanently at the centre of the screen. From trial to trial, a coloured square moved across the bar in a random direction (either one square to the right or to the left). Responses were required to be initiated between 150 to 5000 ms after a square moved one position, otherwise a trial was replaced. The task was self-paced with post-response intervals of 250 ms. In the first block, the moving square was coloured green, and compatible responses were required: children were instructed to press a response button as quickly and as accurately as possible that corresponded to the direction in which the stimulus moved. In the second block, the moving square was coloured red, and incompatible responses were required. The suppression of the automatic compatible response, in order to generate a non-automatic incompatible response, was hypothesized as requiring inhibitory control. In the third block, the colour of the moving square alternated randomly between green and red, and both compatible and incompatible responses were required. Thus, both the direction and the colour of the square were unpredictable. The mixture of both compatible and incompatible trials was hypothesized as requiring high levels of cognitive flexibility in addition to inhibitory control (Los, 1996). The first and second block consisted of 10 practice trials and 40 experimental trials. The third block consisted of 16 practice trials and 80 experimental trials. Administration took about 10 to 15 minutes. The dependent measure was the percentage of errors across blocks, which was the best indicator of endophenotypic vulnerabilities (Rommelse et al, 2007b).

**Time Reproduction**

The Timetest Application Version 1.0 (Barkley, 1998) was used to measure time reproduction. Stimuli consisted of temporal intervals with different durations (4, 8, 12, 16, 20 s) that had to be reproduced as accurately as possible. The task was administered first in the visual modality (light bulb) and thereafter in the auditory modality (tone). Children were not informed about the length of the intervals. In both modalities, 3 practice and 20 experimental trials were
administered. The five interval lengths were randomly presented four times. Task administration for both modalities required 15 minutes. Based on previous results, the main dependent measure was the precision of the reproduction (operationalized as the absolute discrepancy between the response length and the stimulus length) averaged across trials and modalities, which was abnormal in children with ADHD and their non-affected siblings and correlated between siblings (Rommelse et al., 2007a).

Visuo-Spatial Sequencing
The Visuo-Spatial Sequencing task was used to measure accuracy of visuo-spatial working memory (De Sonneville, 1999). Stimuli consisted of nine circles symmetrically organized in a square (3 by 3). On each trial, a sequence of circles was pointed at by a computer-driven hand. Subjects were instructed to replicate the exact same sequence of circles, by pointing to them with the small, self-driven hand. There were no time constrictions. One practice trial and 24 experimental trials were presented. Every succeeding trial increased in difficulty level: an increase in the number of circles required to be remembered and/or an increase in the complexity of the spatial pattern (i.e. the trial consisted of circles that were spatially further removed from one another instead of being close to one another), hence manipulating working memory demands. Task administration took about 7 minutes. Based on previous results, the total number of correct targets in the correct order was used as dependent measure reflecting endophenotypic-like group differences and correlating between siblings (Rommelse et al., 2008a).

Digit Span
The Digit Span forwards and backwards of the WISC-III and WAIS-III (Wechsler, 2000, 2002) were used to obtain an indication of verbal working memory. The forward part required repeating a sequence of numbers in the same order. The backward part consisted of repeating the numbers in the opposite order. Children were instructed to reproduce sequences as accurately as possible. In both parts, one digit was added to the sequence if a child reproduced the sequence successfully. Two practice trials with a 2 digit sequence and (dependent on the child’s performance) a maximum of 9 (forward) or 8 (backward) experimental sequences were administered. Dependent measure was the maximum Digit Span backwards, which proved useful as endophenotypic candidate (Rommelse et al., 2008a).
Chapter 11

**Motor Tasks**

**Pursuit**

This task was designed to measure precision of motor control under continuous adaptation (De Sonnevile, 1999). The stimulus consisted of a randomly moving target (asterisk) that was required to be ‘caught’ by moving a mouse cursor on top of the asterisk. The target moved at a constant speed of 10 mm/s. Children were instructed to ‘catch’ the randomly moving target as precisely as possible. One practice (13 s) and one experimental session (60 s) were administered for both hands separately. Administration took about 5 minutes. The dependent measure was the precision (mean distance in mm between target and cursor calculated per second and averaged across the 60 s experimental session) of the left hand. Previous results have shown that mainly the performance of the left hand was most strongly associated with ADHD (Rommelse et al., 2007c).

**Tracking**

This task aimed to measure precision of motor control without continuous adaptation required (De Sonnevile, 1999). The stimulus consisted of an inner and outer circle (radius 7.5 and 8.5 cm, respectively). Children were instructed to trace an invisible midline (radius 8 cm) between the inner and outer circle as quickly and precisely as possible with a mouse cursor. One practice and one experimental session were administered for both hands separately (clockwise with the right hand and counter clockwise with the left hand). Administration took about 3 minutes. The dependent measure was the precision (mean distance to midline in mm averaged across 60 equal parts of the circle) of the left hand. Previous results have shown that precision of the left hand showed endophenotypic-like characteristics (Rommelse et al., 2007c).

**Tapping**

This task measured speed and variability of self-generated motor output (De Sonnevile, 1999). This task required the child to tap as frequently as possible within a certain time period. During tapping, the number of taps was continuously counted and displayed on the screen. One practice session (5 s) and one experimental session (18 s) were administered for both hands separately. The task was first practised and executed with the index finger of the non-preferred hand, thereafter practised and executed with the index finger of the preferred hand. Administration took about 3 minutes. The dependent measure was the variability (SD of intertap intervals in ms) averaged across hands. Previous results have shown that this measure correlates between siblings (Rommelse et al., 2007d).
Chapter 11

Baseline Speed
This task was designed to measure speed and variability on a simple reaction time task (De Sonneville, 1999). Stimuli consisted of a fixation cross in the centre of a computer screen that changed unpredictably into a white square. Immediately following the response, the white square changed back into the fixation cross. The time interval between a response and the emergence of the next white square varied randomly between 500 to 2500 ms in order to prevent anticipation strategies. Subjects were required to press a key as quickly as possible when the white square appeared. A practice session (10 trials) and an experimental session (32 trials) were administered for both hands separately. The task was first practiced and executed with the index finger of the non-preferred hand, thereafter practiced and executed with the index finger of the preferred hand. Administration took about 5 minutes. Dependent measure was the variability (SD of reaction times in ms) of responses averaged across hands. Previous results have shown that this measure was associated with ADHD and correlated between siblings (Rommelse et al., 2007d).

Motor Timing
This task was designed to measure accuracy and variability of motor timing (Van Meel et al., 2005). In this task a 1 s interval had to be produced. The start of the interval was announced by a tone (80 db, 50 ms). After the subject’s response, visual feedback was given, indicating whether the response was correct, too short or too long. A response was regarded as correct, if it fell between the lower and upper boundary set by a dynamic tracking algorithm. Boundaries were set at 500 to 1500 ms at the beginning of the task. If the response fell within these boundaries, the boundaries for the subsequent trial were narrowed by 100 ms. Likewise, the boundaries of the subsequent trial were widened with 100 ms, if the response on the previous trial fell outside those boundaries. Subjects were instructed to produce as accurately as possible the 1 s interval. Twenty practice trials and 80 experimental trials were administered. Both sessions were preceded by presenting 10 times a cartoon figure for exactly 1 s on the screen to demonstrate the duration of 1 s (Van Meel et al., 2005). Administration took about 8 minutes. The dependent measure was the variability (SD of productions in ms). Previous results have shown that this measure to be a viable endophenotypic candidate (Rommelse et al., 2007d).

Procedure
Testing of children with ADHD and their siblings took place at the VU University Amsterdam or at the Radboud University Nijmegen Medical Centre and was conducted simultaneously for all children in a family. Psychostimulants were discontinued for at least 48 hours before testing took
place (Pelham et al., 1999). Children were motivated with small breaks. At the end of the session, a gift worth approximately € 4 was given. Control children were tested in a similar way in a quiet room at their school. The study had medical-ethical approval.

Data Analyses

Neuropsychological task variables with less than 5% missing data were subjected to expectation maximization to replace the missing data (Tabachnick & Fidell, 2001). The percentage of missing data on the Stop task (9.2%) was considered too large to replace in this manner. Some variables of the neuropsychological tasks were changed in sign, so that the scores of all variables would imply the same meaning: higher scores being indicative of a poor performance. Normalization and standardization of all task variables and an ADHD composite (raw Conners’ DSM-IV Total ADHD averaged across parent and teacher) was performed in SPSS using a Van der Waerden transformation (Statistical Package for the Social Sciences version 14). Correlations were calculated using SAGE (Statistical Analysis for Genetic Epidemiology version 5.3.1). Significance levels were corrected for the non-independency of sib-pairs (i.e. more than one sib-pair per family contributing to the analyses, if the family consisted of three or four children). Alpha was set at 0.05. The following three terms were used: correlation (referring to a correlation between two variables in the same subject), sibling correlation (referring to a correlation between siblings for the same variable), and sibling cross-correlation (referring to a correlation between siblings for two different variables). First, to establish whether the neuropsychological measures were more strongly familially determined than the ADHD composite, sibling correlations were calculated to estimate the degree of familiality of all measures. Sibling correlations for the neuropsychological measures were compared with the sibling correlation for the ADHD composite using dependent correlations one-sided t-tests (Chen & Popovich, 2002). Second, to analyze whether shared familial influences affected both the neuropsychological measures and the ADHD composite, we calculated sibling cross-correlations (neuropsychology of a child with ADHD composite of his/her siblings). An estimation of the shared heritability was performed. Even though it is not possible to estimate reliably heritability coefficients in a sibling-design, since siblings share both half of the additive genetic factors as well as their shared environment, the assumption might be made that sibling similarity is due to additive genetic factors (Andreou et al., 2007), because virtually all twin studies on ADHD have indicated that shared environment does not seem to influence ADHD (Martin et al., 2006a). Heritability coefficients were calculated by multiplying the sibling cross-correlation by two and dividing this score by the correlation between the neuropsychological measure and the ADHD
Chapter 11

composite, as has been done previously (Andreou et al., 2007). Third, in order to investigate whether a neuropsychological composite would provide a better endophenotype instrument than individual task measures, a principal component analysis was run on the ten neuropsychological measures. The composite was subjected to the same analyses as described above and the sibling correlations and sibling cross-correlations were compared between the composite and each neuropsychological measure by means of dependent correlations one-sided t-tests (Chen & Popovich, 2002).

Results

Familiality of Neuropsychological Measures and ADHD Composite

Siblings resembled each other significantly on all neuropsychological measures (with sibling correlations ranging from .14 -.31, Table 11.1) and on the ADHD composite (r = .11, Table 11.1). The sibling correlations for all neuropsychological measures were at least as large as the sibling correlation for the ADHD composite. Six of ten neuropsychological measures revealed sibling correlations that were even significantly larger than the sibling correlation for the ADHD composite (Table 11.1), suggesting these neuropsychological measures showed stronger patterns of familiality (and thus heritability) than the ADHD composite.

Table 11.1 Sibling correlations for the neuropsychological measures compared to sibling correlation for the ADHD composite.

<table>
<thead>
<tr>
<th>Cognitive measures</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>Stop task (stop signal reaction time)</td>
<td>.22 (.12 - .33)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Shifting attention set (% errors)</td>
<td>.23 (.14 - .32)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Time reproduction (absolute deviation)</td>
<td>.25 (.16 - .34)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Visuo-spatial sequencing (N correct targets)</td>
<td>.20 (.11 - .29)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Digit span (backwards)</td>
<td>.18 (.09 -.27)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Motor measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pursuit (precision)</td>
<td>.28 (.19 - .37)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Tracking (precision)</td>
<td>.22 (.13 - .31)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Tapping (variability)</td>
<td>.18 (.09 -.27)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Baseline speed (variability)</td>
<td>.14 (.05 - .23)</td>
<td>.002</td>
</tr>
<tr>
<td>Motor timing (variability)</td>
<td>.31 (.22 - .40)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Note. A = Sibling correlation; B = Sibling correlation for neuropsychological measure compared with sibling correlation for ADHD composite (r = .11 (.02 -.20), p = .02).
Shared Familial (and Heritable) Influences on the Neuropsychological Measures and ADHD Composite

In order for neuropsychological measures to be useful for molecular genetic research, the measures should relate to similar familial (and heritable) influences as the ADHD composite. If this is the case, the neuropsychological measures of a child should correlate with the ADHD composite of his/her siblings (i.e. sibling cross-correlation). Six of ten neuropsychological measures showed such significant sibling cross-correlations, suggesting similar familial factors influenced neuropsychological task performance and the ADHD composite (Table 11.2). On the assumption that shared familiality is entirely due to additive genetic factors (since shared environmental factors do not seem to influence ADHD [Andreou et al., 2007]) heritability estimates were made of the six neuropsychological measures that showed familial overlap with the ADHD composite (2 x sibling cross-correlation / correlation task measure with ADHD composite, Table 11.2). Approximately 62 – 100% of the variance of the six neuropsychological measures was related to heritable factors that also affected the ADHD composite.

Table 11.2 Sibling cross-correlations indicating common familial influences on neuropsychological measures and ADHD composite.

<table>
<thead>
<tr>
<th></th>
<th>Sibling cross-correlation</th>
<th>95% CI</th>
<th>p</th>
<th>% shared heritability with ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop task (stop signal reaction time)</td>
<td>.12</td>
<td>.05 - .20</td>
<td>.002</td>
<td>(.12x2)/.24 = 100%</td>
</tr>
<tr>
<td>Shifting attentional set (% errors)</td>
<td>.05</td>
<td>-.02 -.12</td>
<td>.16</td>
<td></td>
</tr>
<tr>
<td>Time reproduction (absolute deviation)</td>
<td>12</td>
<td>.05 -.19</td>
<td>.001</td>
<td>(.12x2)/.31 = 77%</td>
</tr>
<tr>
<td>Visuo-spatial sequencing (N correct targets)</td>
<td>.08</td>
<td>.01 -.15</td>
<td>.03</td>
<td>(.08x2)/.26 = 62%</td>
</tr>
<tr>
<td>Digit span (backwards)</td>
<td></td>
<td>.06</td>
<td>- .13</td>
<td>.08</td>
</tr>
<tr>
<td>Motor measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pursuit (precision)</td>
<td>.04</td>
<td>-.03 -.11</td>
<td>.23</td>
<td></td>
</tr>
<tr>
<td>Tracking (precision)</td>
<td>.09</td>
<td>.02 -.16</td>
<td>.01</td>
<td>(.09x2)/.27 = 67%</td>
</tr>
<tr>
<td>Tapping (variability)</td>
<td>.08</td>
<td>.02 -.15</td>
<td>.02</td>
<td>(.08x2)/.06 = &gt;100%</td>
</tr>
<tr>
<td>Baseline speed (variability)</td>
<td>.06</td>
<td>-.01 -.12</td>
<td>.09</td>
<td></td>
</tr>
<tr>
<td>Motor timing (variability)</td>
<td>.14</td>
<td>.07 -.21</td>
<td>&lt; .001</td>
<td>(.14x2)/.28 = 100%</td>
</tr>
</tbody>
</table>

Note. Heritability was calculated using the formula: (sibling cross-correlation x 2) / correlation task measure with ADHD composite. Heritability estimates are based on the assumption that sibling resemblance is entirely due to genetic factors (and not shared environmental factors).
Chapter 11

Results of the Neuropsychological Composite

Neuropsychological measures are not free of measurement error. This can negatively influence the results of familiality and heritability. A principal component analysis was run on the variance-covariance matrix to combine all ten neuropsychological measures. One major composite emerged explaining 47% of the variance. The neuropsychological composite correlated significantly between siblings \( r = .34 \) [.23 - .44], \( p < .001 \), which was more strongly than the sibling correlation for the ADHD composite \( t = 4.33, p < .001 \), suggesting the neuropsychological composite to be more familial (and heritable) than the ADHD composite. The neuropsychological composite also showed a significant sibling cross-correlation with the ADHD composite \( r = .14 \) [.06 - .22], \( p = .001 \), indicating similar familial factors influenced the neuropsychological composite and the ADHD composite. If these shared familial factors were complete reflections of additive genetic factors, than 82% (i.e. \( .14 \times 2/34 = 82 \)) of the neuropsychological composite reflected heritable factors that also influenced ADHD.

In order to investigate whether the composite was more suitable as endophenotypic instrument than the individual task measures, the sibling correlations and sibling cross-correlations were compared between the composite and each neuropsychological measure. The sibling correlation using the composite was significantly larger than the sibling correlations for eight of ten single neuropsychological measures \( t \)-values between 1.85 and 3.96 with \( p \)-values between .03 and <.001). Only the sibling correlations for Pursuit and Motor Timing were as large as the sibling correlation for the composite \( t = 1.24, p = .11 \) and \( t = 0.64, p = .26 \), respectively. However, the composite did not have a significant larger sibling cross-correlation with the ADHD composite than most individual task measures \( t \)-values between 0.00 and 1.46 with \( p \)-values between .07 and .50), except for Shifting Attentional Set \( t = 1.70, p = .04 \) and Pursuit \( t = 1.95, p = .03 \). These findings suggest that a neuropsychological composite is overall more strongly familial determined than most individual task measures, though the shared familial overlap with ADHD appears roughly comparable to that of most individual task measures.

Discussion

This study extends previous findings on the utility of neuropsychological measures as candidate endophenotypes for ADHD (Rommelse et al., 2007a, b, c, d, 2008a) by (1) examining whether neuropsychological measures show a stronger degree of familiality than an ADHD composite,
(2) investigating whether similar familial (and heritable) factors influence neuropsychological measures and an ADHD composite, and (3) testing whether a composite of neuropsychological measures is a more powerful endophenotypic instrument than individual task measures.

Results indicated that all neuropsychological measures correlated at least as strongly between siblings as an ADHD composite, with six of ten measures correlating even more strongly between siblings than the ADHD composite. These findings suggest that most neuropsychological tasks, specifically the Stop task, Shifting Attentional Set, Time Reproduction (cognitive tasks) and Pursuit, Tracking, Motor Timing (motor tasks), may be more heritable than the ADHD phenotype itself. This concurs with the concept of (neuropsychological) endophenotypes as being more strongly related to genetic factors (disease genes) than the phenotype (Almasy & Blangero, 2001; Castellanos & Tannock, 2002; Doyle et al., 2005c; Gottesman & Gould, 2003; Rommelse et al., 2007a; Skuse, 2001; Waldman, 2005) and underlines the usefulness of certain neuropsychological measures for detecting underlying familial related dysfunctions. However, some neuropsychological tasks (Visuo-Spatial Sequencing, Digit Span [cognitive tasks] and Tapping and Baseline Speed [motor tasks]) showed comparable degrees of familiarity as the ADHD composite. This may imply that these neuropsychological measures are equally strongly linked to disease genes as the ADHD phenotype and do not form a more powerful instrument to detect these disease genes (Braff & Freedman, 2002). However, they may still prove useful in ADHD molecular research, when they are linked to a smaller number of genes than the ADHD phenotype (Doyle et al., 2005b) and if they facilitate the forming of more homogeneous subgroups of patients having a common neuropsychological deficit.

Further support for the utility of neuropsychological measures was found when the sibling cross-correlations were analyzed: six of ten neuropsychological tasks (Stop Task, Time Reproduction, Visuo-Spatial Sequencing [cognitive tasks] and Tracking, Tapping, Pursuit [motor tasks]) measured in a child were positively and significantly related to the degree of ADHD in his/her siblings. This indicates that similar familial influences have an effect on these neuropsychological measures and the ADHD composite (Andreou et al., 2007; Kuntsi & Stevenson, 2001). Therefore, these neuropsychological measures may facilitate understanding the mode of action of certain risk genes in ADHD: linking a neuropsychological measure to specific genes, may give insight into how these genes act on the ADHD phenotype (Kuntsi & Stevenson, 2001). However, since it is not possible to separate reliably heritable factors from shared environmental factors within a non-twin design, the sibling cross-correlations may also stem from shared environmental influences instead of heritability (Andreou et al., 2007). Still,
since the vast majority of twin studies in ADHD have indicated that shared environment does not seem to play a significant role in determining the degree of ADHD (Faraone & Doyle, 2001; Van ’t Ent et al., 2007), it has been suggested that resemblance between siblings may be entirely due to genetic factors (Andreou et al., 2007). Calculations based on this assumption showed that six of ten neuropsychological measures overlapped genetically with the ADHD composite between 62-100%, making these measures potentially useful for future molecular genetic research in ADHD. The other four neuropsychological measures did not show significant sibling cross-correlations, despite findings of significant sibling correlations. This may suggest that, even though these neuropsychological measures are familial, their familial overlap with ADHD is too weak to be useful as instruments in molecular genetic research of ADHD.

In order to investigate whether a composite of the neuropsychological measures led to an improvement in results, a principal component analysis was run to combine all measures. This neuropsychological composite appeared to be more strongly correlated between siblings (.34) than eight of ten individual measures (between .14-.31), suggesting a neuropsychological composite is overall more strongly familial determined than most individual task measures. However, most sibling cross-correlations (eight of ten) were comparably large between the composite and individual task measures, signalling the shared familial overlap of the composite with the ADHD composite appears roughly comparable to that of most individual task measures. These findings suggest that a neuropsychological composite may prove a more powerful endophenotypical instrument than some individual task measures, possibly because they entail less error variance than individual measures (Rushton et al., 1983), though their power is equivalent to some other individual task measures.

Almost all correlations calculated were small and some were of medium size (Cohen, 1988). This suggests that the familial effects on the neuropsychological measures are modest. However, we did not expect to find large correlations for several reasons. First, it is likely that multiple genes relate to the ADHD phenotype (polygenetically determined disorder), each having a small effect (Faraone & Biederman, 1998) with no single gene being necessary or sufficient to cause ADHD. It was expected that sibling correlations and sibling cross-correlations for familial determined neuropsychological deficits would also be small. Second, previous research on neuropsychological functioning in patients with ADHD has shown that a substantial proportion of patients does not perform abnormally on neuropsychological measures (Nigg et al., 2005), resulting in an overall small association between neuropsychological deficits and ADHD. Nevertheless, in the task battery we administered, six of ten measures and the composite score
showed a higher degree of familiality (and heritability) than the ADHD composite, lending support to the utility of these measures in heritability research of ADHD.

Sibling correlations for the ADHD composite (.11) were lower than one would expect based on previously reported DZ twin correlations on the same ADHD scale (Conners’) (around -.01-.47) (Hudziak et al., 2005; Kuntsi et al., 2000; Martin et al., 2002). There may be several explanations for this. First, in this study the sibling correlation was calculated based on the raw scores (not matched for age and gender) to allow direct comparison with ‘raw’ neuropsychological scores (of which no age and gender scaled scores were available). When the scaled Conners’ score was used, the sibling correlation became larger (.17), yet still somewhat lower than previously reported DZ correlations. An alternative explanation might be that the low correlation was due to the nature of the selected sample studied here (ADHD-families and control-families) in contrast to other (mostly twin) studies which employed population based samples. This might have resulted in a restricted range in ratings and hence, to lower correlations. A third explanation might be that DZ twins are not directly comparable to siblings, since DZ twin correlations tend to be larger than non-twin sibling correlations on a range of cognitive and behavioural measures (Koeppen-Schommerus et al., 2003). This might be related to the fact that DZ-twins are of the same age, whereas siblings are not. It is feasible, therefore, that DZ twins are perceived as more alike than siblings resulting in higher DZ correlations than sibling correlations. In addition to this, being dissimilar in age, siblings will probably be more often rated by different teachers than DZ twins. Correlations between ratings of different observers are generally lower than correlations between ratings of the same observer (Dale et al., 2005).

Limitations
The ADHD composite was obtained by averaging ADHD ratings across parents and teachers to reduce the number of correlations that needed to be calculated and to obtain a more stable and reliable phenotypic measure of ADHD (Conners et al., 2001; Schwarz et al., 1985). However, this may have masked rater-specificity of results. This did not appear to be the case, since none of the sibling correlations and sibling cross-correlations between the neuropsychological measures and parental ADHD ratings on the one hand and between the neuropsychological measures and teachers ADHD ratings on the other hand differed significantly from each other. This is in line with a study showing comparable latent classes underlie Conners’ ratings of parents and teachers (Althoff et al., 2006). However, overall correlations appeared somewhat larger (though not significantly so) when parental ratings of ADHD were used compared to
teacher ratings, which may be related to the fact that siblings are dissimilar in age and, consequently, were often rated by different teachers, which may have introduced extra error variance in the teacher ratings (Dale et al., 2005).

Conclusions
The current study finds support for the hypothesis that neuropsychological measures may aid in the discovery of the genetic underpinnings of ADHD. Results indicated that all ten neuropsychological measures correlated significantly between siblings, indicating familiality of neuropsychological tasks. Six of ten measures appeared more strongly familial than the ADHD composite, suggesting these measures may be more heritable than ADHD. Significant sibling cross-correlations suggested that six of ten neuropsychological measures were related to similar familial (and heritable) factors as the ADHD composite, suggesting these neuropsychological measures to be useful in heritability research in ADHD. An aggregated neuropsychological composite appeared to be the most powerful, since it correlated more strongly between siblings than most individual task measures. These findings suggest heritability research in ADHD will probably be facilitated by including neuropsychological measurements.