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Summary, Discussion, Key Findings, Limitations, Future Directions, and Clinical Implications
ADHD is an impairing neurodevelopmental disorder characterized by inattention, hyperactivity, and impulsivity and is associated with various neurological, neurochemical, neurophysiological, and neuropsychological deviations. The disorder is largely determined by heritable factors (Faraone et al., 2005). However, the identification of risk genes for ADHD by linking chromosomal regions and/or susceptibility genes (the genotype level) to the behavioral symptoms (the phenotype level) has proven to be troublesome, because of the heterogeneity at both levels (Buitelaar, 2005; Gottesman & Gould, 2003). By focusing on neuropsychological dysfunctioning (endophenotypes), which may confer an increased (genetic) risk for ADHD, it may be possible to identify susceptibility genes for ADHD. That is, endophenotypes are considered to be less genetically complex than phenotypes, since they are etiologically ‘closer’ to the disease genes than phenotypes (Almasy & Blangero, 2001; Castellanos & Tannock, 2002; Waldman, 2005). Endophenotypes are statistically more powerful than phenotypes because they are quantitative traits and not diffuse syndromes as in DSM-IV. Endophenotypes may be more useful in exploring different pathways leading to the disorder than phenotypes (Waldman, 2005), because they may form more homogeneous subgroups of patients sharing an underlying deficit.

The overall aim of this dissertation was to examine the viability of neuropsychological measures as endophenotypes for ADHD by studying neuropsychological functions in a large, extensively phenotyped group of participants and adolescents with ADHD and their non-affected siblings from whom DNA was obtained. In addition, control participants were recruited, which resulted in a sample of 816 participants. The focus of the neuropsychological test battery was on two key domains of functioning in ADHD. Firstly on executive functions: inhibition, visuo-spatial and verbal working memory, and set shifting. Secondly on motor functions: speed, variability, control and timing of motor output. It was considered that this battery offered a strong chance of finding endophenotypic dysfunctions.

This dissertation is divided into three parts, in order to cover the primary three research questions. Chapters in Part 1 are all related to the research question of which neuropsychological functions formed candidate endophenotypes. It was studied whether a broad range of neuropsychological functions fulfilled several key characteristics of an endophenotype, such as whether participants with ADHD were on average impaired compared to control participants, whether comparable deficits were also present in the non-affected siblings, and whether siblings resembled each other on neuropsychological functioning. The chapters in Part 2 of this dissertation aimed at examining the research question of how the endophenotype and phenotype are related and what factors may influence this relationship. It
was tested whether group differences were equal in magnitude at the endophenotypic and phenotypic levels, and whether the relationship between endophenotype and phenotype was mediated and/or moderated by factors such as IQ, age, gender, and rater bias. It was also studied whether ADHD endophenotypes were related to comorbid disorders, and whether neuropsychological endophenotypes were more strongly linked to heritable factors than the ADHD phenotype itself. The chapters in Part 3 focused on the research question of whether neuropsychological functions were related to specific genes and chromosomal regions. The relationship between selected candidate genes (DAT1, DRD4, MAOA) and neuropsychological functions was explored, as well as the relation between chromosomal locations and neuropsychological functions.

Summary

Part 1

In Chapter 2, the most intensively studied aspects of executive function (EF) in ADHD (inhibition, visuo-spatial and verbal working memory) showed endophenotypic characteristics: EF deficits were not only present in participants with ADHD, but were observed also in their at-risk siblings. Similar results were found for Verbal IQ. Performance IQ appeared (nearly) normal in affected and non-affected siblings. Interestingly, most results indicated an independent segregation of EF and IQ deficits: Correlations and sibling cross-correlations were not significant between EF and IQ. Group effects in EF could not be explained by group differences on IQ and vice versa. Siblings resembled each other in their EF-IQ discrepancy instead of having generalized impairments across both domains: Siblings of probands with EF (but not IQ) problems displayed the same selective EF (but not IQ) deficit. In contrast, the three EF functions appeared to segregate together, as did Verbal and Performance IQ. Thus, it was concluded that EF and IQ impairments segregate relatively independently of each other.

Results reported in Chapter 3 revealed that time reproduction, a function strongly related to both inhibition and working memory, was impaired in affected participants and their non-affected siblings and correlated between siblings. As expected, longer test durations discriminated both groups even more from controls, suggesting that increasing demands on the ability to reproduce time made the performance deficit more prominent. Unexpected was the moderating influence of age on group difference for time reproduction. Participants with ADHD could be clearly dissociated from control participants up to the age of 9. After that age, group
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differences were somewhat attenuated, although still present. Differences between non-affected siblings and controls were constant across the age range studied. These findings suggested that time reproduction may serve as a candidate endophenotype for ADHD, predominantly in younger children with (a genetic risk for) ADHD.

Chapter 4 illustrated that deficits in inhibition and visuo-spatial working memory may also be revealed using eye tracking paradigms, reflected by an increase in anticipatory saccades and impaired accuracy in memory-guided saccades, respectively. Intriguing was the finding of a tendency to undershoot the memorized location: Normal controls tended to undershoot the memorized location (which has been reported previously in healthy participants). Participants with ADHD and their non-affected siblings showed this tendency to a lesser degree, which resulted in an overshoot of the memorized target in affected participants. This tendency to overshoot saccades relative to controls was not related to the measures of inhibition and visuo-spatial working memory, suggesting that neuropsychological endophenotypes may also be found outside the spectrum of the frequently studied EF domain. It was concluded that memory-guided saccade deficits may relate to a familial predisposition for ADHD.

Endophenotypes outside the EF domain were found in the following chapters. In Chapter 5, participants with ADHD and their non-affected siblings committed more errors than controls on a baseline measure of responding (i.e. simple reaction time task). Moreover, compared to their baseline speed and accuracy of responding, participants with ADHD and their non-affected siblings were not disproportionately slower or more inaccurate, when demands for motor inhibition or cognitive flexibility were added to the task. This suggested that poorer performance on EF tasks in participants with ADHD and their non-affected siblings may result from deficiencies in lower order cognitive processes (such as motor functioning) and not (only) from higher order cognitive processes/executive functions.

Additional evidence for endophenotypes outside the EF domain, but within the motor domain, was found in the following two chapters. In Chapter 6 the accuracy and variability of motor timing were studied. Two basic motor tasks were administered alongside the motor timing task to account for generalized deficiencies in speed and variability of motor output. Variability of motor timing convincingly met all required characteristics of an endophenotype, though accuracy (tendency to under-produce the time interval) appeared predominantly present in affected participants and not in non-affected siblings. Interestingly, slow and variable motor output without a timing component was only present in affected participants and not in non-affected siblings, suggesting that slow and variable basic motor output is not convincingly associated with a familial vulnerability for the disorder. In addition, self-generated motor output
was normal in affected participants (and non-affected siblings). This suggests that the other two tasks may have required some cognitive processing (e.g. registering a stimulus and responding to it), whereas self-generated motor output only required executing a motor action. The findings in this chapter suggested that abnormalities in motor timing were predominantly related to deficient motor timing processes and not to a generalized deficient motor functioning.

Chapter 7 attempted to replicate previous findings of abnormal higher-order controlled motor deficits in participants with ADHD and their non-affected siblings (Slaats-Willemse et al., 2005b). Like Slaats-Willemse and colleagues, we found that participants with ADHD were less precise and stable in their motor control. However, findings differed with respect to the non-affected siblings; Slaats-Willemse et al. reported non-affected siblings to be selectively impaired in motor control requiring continuous adaptation, whereas we found non-affected siblings to be selectively impaired in motor control requiring following a known pathway. In addition, the findings here suggested that group differences were moderated by an effect of hand not reported by Slaats-Willemse et al.: No group differences emerged, when the right hand was used. Group differences did emerge when the left hand was used. This finding was possibly related to the known right hemispheric brain pathology in participants with ADHD and/or to differential effects of daily practice with both hands. Imprecision and instability of motor control of the left hand appeared to be useful as an endophenotype.

The final chapter of Part 1, Chapter 8, tested whether possible alterations in somatosensory functioning (i.e. processing of tactile and kinesthetic stimuli) in ADHD, could be found in non-affected siblings, hence supporting the viability of non-EF endophenotypes. Further, the subjective experience of pain was assessed. Results suggested that tactile perception, but not kinesthesia, was deviant in participants with ADHD and their non-affected siblings. Only non-affected siblings, but not affected participants, reported a lower level of emotionality and intensity of previously experienced pain compared to controls. The ‘objective’ tests of somatosensory functioning did not relate to the subjective sensation of pain. These findings suggested that alterations in tactile perception may relate to a familial susceptibility for ADHD. Furthermore, clinicians should be aware of possible under-reportage of experienced pain in siblings of children with ADHD.

Part 2
In Chapter 9 several issues were investigated. First, the predictive validity for the ADHD diagnosis was assessed of the endophenotype of an aggregated component score combining ten neuropsychological task measures. The endophenotypic construct classified children with
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moderate accuracy (about 50% classified correctly in each of the three groups), suggesting substantial overlap between endophenotypic functioning in the groups of affected children, non-affected siblings, and controls. A second issue studied was whether group differences at the endophenotypic and phenotypic level were comparable in their magnitude. This was the case, when non-affected siblings were compared to controls. It was found that their subtle neuropsychological deviations were proportionally related to subtle behavioral deviations. However, group differences differed in magnitude, when affected participants were compared to controls: Affected participants displayed a more severe phenotype than endophenotype. This suggests that other factors aggravate the ADHD symptoms in participants. A third topic was whether the relation between endophenotype and phenotype was mediated and/or moderated by gender, age, IQ, and rater bias. A potentially moderating effect (age) was found as well as several mediating effects (gender, age, IQ). However, none of the effects studied could account for the finding that affected participants had a more severe phenotype than endophenotype. These findings suggest that neuropsychological endophenotypes are moderately predictive of ADHD diagnosis, but other factors may aggravate ADHD symptoms in affected participants. In addition, the relationship between endophenotype and phenotype is similar for boys and girls, both when ADHD ratings are made by parents or teachers, and across the IQ range. This relation is probably similar in the age range of 5 to 19 years.

Chapter 10 focused on the relationship between the ADHD-endophenotype of an aggregated component score combining ten neuropsychological task measures and comorbid disorders. ADHD was strongly related to oppositional defiant behaviors, followed by autistic traits, and succeeded by motor coordination problems, anxiety, and reading problems. Importantly, the neuropsychological composite ADHD-endophenotype was related to the comorbid problems and cross-related to comorbid problems in siblings, even when correcting for the presence of ADHD. This may point to a shared etiology of ADHD and comorbid problems. Further support for this interpretation is given by the fact that ADHD in combination with a comorbid problem did not appear to be a distinct subtype of ADHD, but rather ‘more of the same’ of both disorders.

A final issue examined in this section was whether neuropsychological functions as endophenotypes were more useful than phenotypic measures of ADHD with respect to the familiality of the disorder. This was tested in Chapter 11. Results revealed that six of the ten neuropsychological measures correlated more strongly between siblings than an ADHD composite, suggesting that these measures may have a larger heritability than ADHD symptomatology itself. Furthermore, significant sibling cross-correlations also suggested that six
of the ten neuropsychological measures were related to similar familial (and heritable) factors as ADHD, thus indicating 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 individual task measures. These findings suggest that genetic research in ADHD may be facilitated by including neuropsychological functions.

**Part 3**

Chapter 12 attempted to shed light on the mechanisms of DAT1 (the gene coding for dopamine transporter 1) effects on ADHD by reviewing previous studies linking this gene to neurophysiological and neuropsychological functions and by studying the relation of DAT1 with neuropsychological functions collected in this dissertation. The review indicated that the majority of studies did not find a relation between DAT1 and neurophysiological and neuropsychological measures. In the Dutch IMAGE sample, several of the polymorphisms of DAT1 were associated with ADHD, and ADHD was associated with impaired neuropsychological functioning. However, none of the DAT1 polymorphisms was convincingly associated with neuropsychological dysfunctioning. This suggested that the effect of DAT1 on ADHD was not mediated by neuropsychological functions as assessed here. It may, however, be hypothesized that DAT1 has an effect on neuropsychological processes not examined in our study or previous studies, such as delay aversion, since DAT1 is mainly expressed in the striatum and not in the prefrontal cortex. Hence, DAT1 may influence striatum related functions (such as delay aversion and motor functions) more heavily than prefrontal related functions (such as executive functions). Some support for this hypothesis was found in the more detailed analysis of individual neuropsychological tests: If there was any association between DAT1 and neuropsychological functioning in our sample, it was within the domain of motor functioning and not within the executive domain (however, most of these associations did not survive correction for multiple testing). Interestingly, associations between DAT1 with ADHD were only found in adolescents, which may suggest that DAT1 mainly exerts its effect in adolescence, and/or that having a more persistent form of ADHD may mark a more severe or homogeneous genetic form of the disorder.

Chapter 13 illustrated that DRD4 (the gene coding for dopamine receptor D4) was associated with overall neuropsychological functioning, but this association was reversed in adolescents and children; specific for non-affected siblings and not affected participants; and robustly carried by cognitive functioning and not by motor functioning. In children, the 7-repeat allele of the exon polymorphism of DRD4 was related to better cognitive functioning, whereas
the reverse was true in the adolescents. These results suggest that the effect of the DRD4 7-
repeat allele on neuropsychological functioning is dependent on age and ADHD status and
robustly carried by cognitive and not motor functioning. The latter finding may be explained by
the fact that DRD4 is mainly expressed in the prefrontal lobes (strongly related to
executive/cognitive functions) and not, or to a lesser extent, in other parts of the brain underlying
motor functioning.

A third candidate gene that was studied in relation to neuropsychological functioning
measures was MAOA (coding for a mitochondrial enzyme involved in the pre-synaptic
degradation of monoamines [monoamine oxidase A]). Results are described in Chapter 14. It
was hypothesized that sex differences for ADHD might be related to genes on the X-
chromosome, like the MAOA gene. That is, unlike girls, boys do not have a spare X-
chromosome, making the effect on an X-linked gene on cognition and behavior possibly
stronger in boys compared to girls. Analyses were conducted using a haplotype based on three
single nucleotide polymorphisms. Two haplotypes (GGC and ATT) captured 97% of the genetic
variance in the investigated MAOA SNPs. The ATT haplotype was more common in non-
affected siblings, conferring a protective effect for ADHD in both boys and girls. The ATT
haplotype was associated with poorer motor control in boys, but with better visuo-spatial
working memory in girls, suggesting the target and direction of the MAOA effect on
neuropsychological functioning may be different in boys and girls. These differences were
hypothesized as relating to many biological differences between males and females in serotonin
neurotransmission. MAOA mainly influences the metabolization of serotonin; similar serotonin
levels may produce opposite behavioral and cognitive effects. It is thus feasible that the effect of
MAOA (through serotonin levels) on neuropsychological functions may not necessarily be
comparable between boys and girls with ADHD. These findings suggest the genetic and
neuropsychological mechanisms underlying ADHD may be different in boys and girls and
underlines the importance of taking into account sex effects when studying ADHD.

Using a whole-genome QTL linkage analysis in Chapter 15, effort was made to identify
chromosomal regions possibly conferring risk genes for ADHD. Two genome-wide significant
linkage peaks were found, one for Motor Timing on chromosome 2q21.1 (LOD score: 3.944)
and one for Digit Span on 13q12.11 (LOD score: 3.959). Ten suggestive linkage peaks were
found (LOD scores ≥ 2) on chromosomes 2p, 2q, 3p, 4q, 8q, 12p, 12q, 14q, 17q. The suggestive
linkage signal for the component score that was found at 2q14.3 (LOD score: 2.878) overlapped
with the region linking to Motor Timing. Several candidate genes were located in the region of
the two significant linkage signals, which may prove useful in further association analyses.
Several of the linkage peaks overlapped with previously reported linkage signals for reading disability and autism, possibly pinpointing to pleiotrophic loci that relate to neuropsychological deficits seen in both patients with ADHD and reading disability and/or autism. Some overlap was present in linkage signals with previous studies using cognitive measures in linkage analyses. In conclusion, our results suggest that neuropsychological candidate ADHD-endophenotypes may aid in the discovery of novel ADHD genes through linkage analysis.

Discussion
With respect to the main research questions of Part I of this dissertation, multiple neuropsychological constructs appeared useful as candidate endophenotypes. Inhibition, visuospatial and verbal working memory, verbal intelligence, time reproduction, motor timing, motor control, oculomotor control, and the processing of tactile stimuli all fulfilled the required characteristics of an endophenotype. These findings give rise to several points of discussion. First, findings suggest that affected and non-affected participants differ quantitatively, but not qualitatively, in almost all investigated measures, and that ADHD-related dysfunctions can be found in a widespread area of functioning, ranging from executive functions, motor functions, to the basic processing of tactile stimuli. This hypothesis is supported when the distribution of abnormally performing children is displayed (Figure 16.1): The shape of the distributions is similar for the three groups. The finding that affected and non-affected siblings differ quantitatively, but not necessarily qualitatively, is in line with numerous previous studies reporting ADHD to be the extreme end of a continuum (Chen et al., 2008; Kalff et al., 2003, 2005; Polderman et al., 2007).

A second point is that non-affected siblings performed normally on several measures of motor functioning on which their affected siblings performed abnormally, e.g. speed and variability of motor output on a simple reaction time task (Chapter 6) and precision and stability of motor control during pursuit (Chapter 7). This is in line with a previous study of Durston et al. (2004), in which they reported that non-affected siblings had smaller prefrontal lobe volumes (like affected children), but normal cerebellar volumes (unlike affected children). Given that executive functions are closely linked to prefrontal lobe functioning and motor functions to cerebellar functioning, it may be suggested that some motor dysfunctions are merely associated with ADHD (i.e. caused by the presence of ADHD itself), or related to risk factors unique to the affected child (Durston et al., 2004). In the latter case, some motor
dysfunctions may aid in understanding why certain siblings do, but others do not develop ADHD.

**Figure 16.1** Distribution of (ab)normally performing affected children, non-affected siblings, and controls when a cut-off was chosen at the 10\textsuperscript{th} percentile of worst performing control children (Nigg et al., 2004).

Moreover, executive functions appear more viable than motor functions as endophenotypes of ADHD. Given that endophenotypes are proposed to reflect an underlying susceptibility to a disorder (Gottesman & Gould, 2003), it is of importance that (genetically) at-risk non-affected siblings display an intermediate position between affected participants and controls. This was true for all executive functions on which the affected siblings deviated from controls, but not for all motor functions. Furthermore, when five executive tasks and the five motor tasks were combined to form an executive and a motor component, the executive component discriminated non-affected siblings better from controls than the motor component (*Addendum Chapter* 9).

A fourth discussion point relates to the findings of Part 1: At first sight, the conclusions from *Chapters 2 and 5* appear contradictory. In *Chapter 2* it was concluded that multiple executive functions (inhibition, visuo-spatial and verbal working memory) were candidate endophenotypes. In contrast, in *Chapter 5* it was concluded that poorer performance in inhibition
and cognitive flexibility in participants with ADHD and their (non)affected siblings may result from deficiencies in lower order cognitive processes (such as motor dysfunctions) and not (only) from higher order cognitive processes. We believe the explanation for this discrepancy lies in the different types of tasks used in both chapters. In Chapter 3, the Stop task was used to measure inhibition, in which participants are required to withhold a prepotent response on some trials, whereas participants had to execute a response on every trial on the Attentional Set Shifting task used in Chapter 5. Withholding a response on some trials (Chapter 2) or withholding an automatic/compatible response on all trials while executing a controlled/incompatible response (Chapter 5) may rely on different cognitive processes, which may be differentially impaired in ADHD. Similarly, cognitive flexibility as assessed in previous studies using the Wisconsin Card Sorting test, might rely on different processes than that assessed by the Attentional Set Shifting task: The Wisconsin Card Sorting test requires a subject to extract the problems solving rules, which also change during the test without the subject’s knowledge, whereas in the Attentional Set Shifting task, the problem solving rule is already known to the subject and constant during the test. Therefore, the results in these two chapters should not be considered as contradictory, but merely pointing to the fact that different processes may underlie constructs such as inhibition and flexibility (Shiffrin & Schneider, 1977). Combining the findings of both chapters suggests that ADHD may be characterized by inhibitory deficits when inhibitory control is needed unexpectedly, but not when the child knows beforehand what he/she needs to inhibit. Furthermore, normal performance was found on an oculomotor task measuring reflexive suppression of task-irrelevant stimuli (Van der Stigchel et al., 2007). Taken together, patients with ADHD may be characterized by inhibitory deficits limited to unexpected situations in which the stimulus is perceived as relevant to them. In other situations, for example, when they know beforehand that inhibitory control is required and/or when the to-be-inhibited stimulus is not perceived as relevant to them, patients with ADHD may display a normal level of inhibitory control.

Findings of Part 2 are now discussed, concerning the relationship between the ADHD-endophenotype and the ADHD-phenotype. In Chapters 9 and 10 a principal component analysis was performed on the ten main tasks (five executive and five motor tasks). For the analyses, one dependent variable of each of these ten tasks was used that gave the best results in endophenotypic analyses. A single construct emerged on which all ten tasks loaded. This is noteworthy, given that the unit of measurement of the dependent variables of the tasks varied greatly: reaction time in milliseconds, standard deviation of reaction time in milliseconds,
number of correct answers, % errors, deviation in millimetres, total absolute deviation in seconds. It could be argued that all variables related to a single construct because all ten variables were normalized and standardized, hence the single construct is due to the scale of measurement (z-scores). However, a principal component analysis on the raw data revealed exactly the same results (i.e. a single component solution) (Chapter 9). It thus appears that all tasks relate to one underlying construct, and no strict dissociation can be made between executive and motor functioning tasks. The brain works as an integrated entity: Even though we may distinguish between different function domains based on theoretical grounds, the data suggest that all functions are related to each other. Nevertheless, combining the executive and motor tasks to an executive and motor component, revealed that the executive component was related to IQ, but not the motor component (Addendum Chapter 9), providing some support for the distinctiveness of executive and motor functioning.

In Chapter 9, it was shown that non-affected siblings deviate from controls both in their behaviour as well as in their overall neuropsychological functioning. This does not imply that non-affected siblings were behaviorally abnormal (i.e. showed a subclinical form of ADHD): The mean T-scores on the ADHD symptomatology scales of the Conners were far below clinical threshold and all were below the mean of the norm scores (< T = 50). We believe that combining all ten task measures into one construct may result in the overall picture of subtle neuropsychological abnormality equivalent to subtle behavioral abnormality as presented in Chapter 9, but on some individual task measures the discrepancy between non-affected siblings and controls is larger than on behavioral measures, suggesting these task measures to be more sensitive to increased genetic susceptibility for ADHD than ADHD phenotypic measures.

In addition to the above discussion point, the question may be asked why the group of non-affected siblings did not differ significantly from the control group on the ADHD measures in the chapters in the first part of this dissertation, while non-affected siblings did differ significantly from the same group of controls in Chapter 9. This can be explained by differences in analyses in the chapters in the first part of this dissertation compared to the analyses described in Chapter 9. In the former, group differences were examined on several individual ADHD measures (inattention, hyperactivity-impulsivity and ADHD total for both parents and teachers), whereas in the latter an ANOVA was performed on one ADHD composite measure (combining all ADHD measures). This latter measure may be a more robust measure of ADHD symptomatology encompassing less error variance than the individual measures, hence resulting in subtle group differences between non-affected siblings and controls not detected using individual ADHD measures.
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Another issue that emerges from Part 2 is whether or not age moderates the relation between neuropsychological dysfunctions and ADHD. Previous studies have shown that the severity of ADHD may decline with age (Faraone et al., 2000c, 2006; Willoughby, 2003) as may the associated structural brain abnormalities (Shaw et al., 2007). Some support for the moderating influence of age was found for time reproduction (Chapter 3) as well as for the overall measure of neuropsychological functioning (Chapter 9). In Chapter 3, the moderating effect of age was related to the affected participants: Younger participants with ADHD deviated more strongly from younger controls with respect to time reproduction than older affected participants from older controls. In Chapter 9, the moderating effect of age was related to the non-affected siblings: Younger non-affected siblings deviated more from younger control participants with respect to overall neuropsychological functioning than older non-affected siblings from older controls. However, this latter effect was only significant in one half of the sample and non-significant in the other half. Importantly, no moderating effect of age was found in the other chapters. The overall picture suggests that age has a strong influence on both neuropsychological functioning and ADHD (both improve with age), but affected (and non-affected) participants do not seem to ‘catch up’ with control participants in their neuropsychological functioning at an older age. However, this needs to be validated using a longitudinal design instead of a cross-sectional design as has been done in this dissertation.

A fifth point concerning Part 2 is how the findings presented in this dissertation should be seen in relation to comorbid disorders. As described in previous studies (Bauemeister et al., 2007; Cunningham & Boyle, 2002; Gillberg et al., 2004; Jensen et al., 1997), ADHD is frequently associated with other disorders. It has been proposed that ADHD-endophenotypes should be specific to ADHD and not related to other disorders (Skuse, 2001). However, others have argued that it is unlikely that ADHD-endophenotypes are unrelated to comorbid disorders, because similar risk genes may underlie ADHD and comorbid disorders (Banaschewski et al., 2005; Coolidge et al., 2000; Dick et al., 2005; Nadder et al., 2002). The findings in Chapter 10 provide support for the latter hypothesis. An aggregated ADHD-endophenotype was related to multiple comorbid disorders, even when analyses were corrected for the presence of ADHD.

From Part 4 two issues emerge, the first being the mediation by neuropsychology and age in relation to candidate genes. The viability of neuropsychological functions as mediating vulnerability traits between genotype and phenotype may seem questionable after reading Chapter 12. The DAT1 gene was associated with ADHD, but not with impaired neuropsychological functioning. We suspect that DAT1 may influence specific
neuropsychological processes not examined in our study (such as delay aversion), since DAT1 is mainly expressed in the striatum and not prefrontal cortex (Diamond, 2007; Durston et al., 2005; Schott et al., 2006). Support for the viability of neuropsychological functions as mediating vulnerability traits between genotype and phenotype emerged from Chapter 13. The DRD4 gene was associated with four of five executive tasks, supporting the functionality of the DRD4 gene in prefrontal cortex related functions. In addition, the MAOA gene (Chapter 14) was associated with motor control in boys and with visuo-spatial working memory in girls. Additional support for the use of neuropsychological functions in heritability research of ADHD was provided in Chapter 15, where two genome-wide significant linkage peaks were found using neuropsychological measures, and several suggestive linkage signals were detected.

A second point for consideration is the moderating effect of age with respect to the effect of candidate genes on neuropsychological functioning and ADHD diagnosis. Both in Chapters 12 and 13, the effect of a dopaminergic candidate gene was not comparable in the child (< 11.5 years) and adolescent (>11.5 years) subsamples. In Chapter 12, the effect of DAT1 on ADHD was only present in the adolescents and not children, and the few associations between DAT1 and neuropsychological measures were attributable to the adolescent group. In Chapter 13, the effects of DRD4 on ADHD and neuropsychological functioning were completely reversed: The 7-repeat allele was associated with lower levels of ADHD and better neuropsychological functioning in the children, yet associated with higher levels of ADHD and poorer neuropsychological functioning in the adolescents. This suggests that the effect of (certain) dopaminergic genes on ADHD is not constant across development, but becomes apparent in late childhood and adolescence. This may be related to the finding that dopamine levels decrease with increasing age, resulting in a relatively larger effect of ‘abnormal’ dopamine genes on ADHD. Some support for this hypothesis has also been reported by Barkley et al. (2006). They followed children through to adulthood and reported that the effect of DAT1 on phenotypic measures of ADHD increased substantially with increasing age. Given that the genotype did not differ between measurement points in childhood, adolescence, and adulthood, the study of Barkley et al. (2006) provides preliminary evidence that the effect of DAT1 on ADHD may be stronger in older subjects with ADHD, than in younger ADHD participants. Additional indirect support for the hypothesis that the moderating effect of age is specific for dopaminergic genes was found in Chapter 14, where age did not have a moderating effect on the results of the MAOA gene, a gene more strongly related to the metabolization of serotonin than of dopamine. However, an alternative explanation is also possible. It may be that
adolescents and adults with ADHD carry a stronger genetic load or form a genetically more homogeneous subgroup of ADHD patients than preadolescent participants with ADHD. That is, having a persistent form of ADHD, that continues into adolescence and adulthood, may be more strongly related to genetic factors than a remitting form of ADHD. Together with the possibility of a moderating effect of age on the relationship between neuropsychological functioning and ADHD, it seems vital to take age effects into account when studying genotype – endophenotype – phenotype relationships in ADHD.

A third discussion point relates to the differences in heritability estimates of the neuropsychological measures presented in Chapter 11 and Chapter 15. The heritability estimates presented in both chapters are not directly comparable, due to several methodological differences. First, in Chapter 11, heritability estimates were simply based on a doubling of sibling correlations assuming sibling resemblance is entirely due to genetic factors and not shared environmental factors. In Chapter 15, however, heritability estimates were estimated using the more accurate SOLAR software, which takes into account familiar relationship and trait distribution among affected and non-affected individuals. Second, heritability estimates in Chapter 11 were based on the whole sample (both ADHD- and control-families), whereas in Chapter 15 heritability estimates were based on controls only. Third, heritability estimates in Chapter 11 represent the shared heritability with the ADHD phenotype, whereas heritability estimates in Chapter 15 were calculated independent of ADHD.

**Key findings**

- A large array of neuropsychological functions (ranging from executive functions to motor functions and to the processing of tactile stimuli) form candidate ADHD endophenotypes, hence supporting the role of neuropsychological functions in heritability research of ADHD;
- Executive functions appear somewhat more viable as ADHD endophenotypes than motor functions;
- Nevertheless, executive and motor functions are interrelated and problems in motor functioning may sometimes explain poor performance on tasks aimed to measure executive functioning;
- On the majority of neuropsychological and phenotypical measures, non-affected siblings differ quantitatively, but not qualitatively, from their affected siblings, supporting the hypothesis that ADHD forms the extreme end of a continuum;
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- Group differences at the endophenotypic and phenotypic levels are equal in magnitude for non-affected siblings and controls, but unequal for affected participants and controls: Affected children display a more severe phenotype than endophenotype, suggesting that other factors aggravate the ADHD symptoms in affected participants;
- The relationship between endophenotype and phenotype is similar for boys and girls, similar when ADHD ratings are made by parents and teachers, similar across the IQ range, and probably similar across the ages 5 to 19;
- ADHD endophenotypes also relate to comorbid disorders, possibly indicating a shared etiology of ADHD and comorbid problems;
- Some neuropsychological measures are more familial than measures of ADHD, making them useful tools in genetic research of ADHD;
- The effect of dopamine genes appears not comparable in children and adolescents, with reversed or stronger effects in adolescence than childhood ADHD;
- DAT1 has no effect on executive functions, but may have a small effect on motor functions, whereas the reverse is true for DRD4;
- The effect of the X-linked gene MAOA on neuropsychological functioning is not comparable in boys and girls, suggesting that the genetic and neuropsychological mechanisms underlying ADHD may be different in boys and girls;
- Using neuropsychological functions, two genome-wide significant linkage peaks and several suggestive linkage peaks are identified, possibly aiding in the discovery of novel ADHD genes.

Limitations

Some limitations of the studies presented in this dissertation should be noted. First, the participants varied widely in age (5-19 years). Since age has a strong effect on neuropsychological functioning and ADHD, the age effect may have masked other more subtle effects of, for example, group differences on neuropsychological measures. However, we controlled for age-effects by matching the groups for age, by always covarying for age in analyses, and by testing whether the effect of age on neuropsychological functions was comparable across groups. The wide age range may be considered a strength of the study, since it allowed us to generalize results across development. Nevertheless, the optimal approach for studying the effects of age is the use of a longitudinal design.
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Second, boys were overrepresented in the ADHD group, whereas they were underrepresented in the control group. This was due to the fact that ADHD is more frequently diagnosed in boys than in girls and because healthy girls are apparently more willing to participate in research than healthy boys. However, we do not believe that this has affected the results, since the effect of gender was analysed and, when necessary, accounted for in each study. In the majority of studies, gender did not have an effect neuropsychological functioning.

Third, we did not administer the PACS interview to non-affected siblings. This might have resulted in undetected ADHD cases in the non-affected sibling group, which in turn might explain the neuropsychological deficits observed in this group. However, this explanation is unlikely, since all siblings were thoroughly screened with questionnaires and if they scored clinically on any of the screeners, the PACS interview was indeed administered. Sub-clinical siblings were classified as affected in order to rule out false negative diagnoses in the non-affected siblings group, making the endophenotypic analyses more stringent.

An arguable fourth limitation is that we did not determine whether the various neuropsychological functions were indeed heritable, which is a required criterion for an endophenotype (Gottesman and Gould, 2003). However, we did establish that all measures were familial. Since shared environment does not seem to play an (important) role in ADHD, the familiality of the measures is most likely attributable to genetic effects (Faraone et al., 2005).

Fifth, we did not take into account environmental risk factors (such as pre- and perinatal complications, smoking of the mother during pregnancy, socio-economic-status, et cetera). Therefore, we do not know how these factors mediated and/or moderated the findings presented in this dissertation. Data on environmental factors was gathered though, and will be analyzed in relation to the neuropsychological tasks in the near future. It is not expected that effects of environmental factors will change the results as presented in this dissertation, given that ADHD is for more than 70% influenced by genetic factors (Faraone et al., 2005). However, environmental factors may shed light on the finding of a disproportionate severe phenotype compared to endophenotype in affected participants (Chapter 9) and may have played a role in interacting with genetic risk factors.

Sixth, the neuropsychological measures used here are by no means representative of the full domain of neuropsychological functions and tasks relevant for ADHD.

A final limitation may be that only subjects of Caucasian ethnicity were included in the study, in order to minimize genetic variation between families. However, this may limit the generalization of our findings to other ethnic groups.
Chapter 16

Future Directions

We aim to study longitudinally the sample described in this dissertation. This will offer several opportunities to further understand the etiology and nature of ADHD. The first aim of the follow-up may be to examine the utility of structural and functional MRI measures as endophenotypes by comparing affected participants, their non-affected siblings and controls on these measures, to test the resemblance between siblings on these measures, and by linking these measures to the available DNA data. Although more time consuming and costly than neuropsychological measures without imaging techniques, MRI measures may have several benefits over neuropsychological measures. It has been proposed that MRI measures may be more strongly linked to disease genes and have a less complex aetiology than neuropsychological functions, because they are even closer to the biological basis of disease than the neuropsychological functions (Gottesman & Gould, 2003). Consequently, MRI measures may be more powerful for linkage and association techniques than the neuropsychological tasks without imaging techniques. Furthermore, fMRI measures may be more sensitive than neuropsychological tasks in detecting subtle underlying functional abnormalities, since several studies have documented an abnormal brain activation during performance of interference control and working memory tasks, while task performance was normal (Busch et al., 1999; Valera et al., 2005).

A second aim of the follow-up may be to extend the neuropsychological and phenotypical data on participants with comparable data on the parents of ADHD-families. Such data were not collected in the original IMAGE sample, but DNA is available for these parents. Data on parental neuropsychological functioning and phenotypic characteristics are useful for several reasons. They can be used to increase the power of family-based association tests by incorporating the parental neuropsychological characteristics and phenotypes into the test statistic. Of equal importance, parental data can be used to stratify the cohort into families that show stronger versus weaker familial transmission of ADHD and associated neuropsychological deficits. Furthermore, adding parental data to the cohort may shed light on the trans-generational familial transmission of ADHD and associated neuropsychological deficits and may add to the (still limited) existing knowledge on ADHD in adults, since it is expected that a substantial proportion of the parents will have (sub-clinical) ADHD themselves (Faraone et al., 1997).

A third aim of the follow-up may be to investigate the longitudinal aspect of neuropsychological deficits and ADHD symptom severity in participants with ADHD and their
siblings. Few studies have investigated the longitudinal aspects of neuropsychological functioning and ADHD severity in patients with ADHD and no longitudinal studies are available on these aspects in siblings. This will enable us to investigate the developmental effect on our data and its possible moderating influence on neuropsychological functioning and ADHD. Using a longitudinal design, we will be able to determine which neuropsychological processes are potentially stable traits versus state fluctuations and relate this distinction to both familial effects and molecular genetics. In addition, this may shed light on the predictive value of neuropsychological functioning for future phenotypic characteristics.

**Clinical Implications**

In general, neuropsychological testing in addition to standard methods of assessment may support clinical practice by providing a cognitive profile with weaknesses and strengths of the child. That is, a child may have a particularly weak working memory, and may be helped with working memory training (Klingberg et al., 2005) and/or external reminders. In addition, recurrent testing of a child during his/her development allows studying his/her cognitive development and possible progress during treatment. Neuropsychological functions are, however, not accurate enough to be useful for diagnostic purposes. Even though results in this dissertation suggest that affected participants and controls can be distinguished from each other with moderate accuracy, there is substantial heterogeneity in both groups (i.e. affected participants having a normal neuropsychological profile and controls having an abnormal one). Results in this dissertation suggest that neuropsychological vulnerabilities may be present in siblings of participants with ADHD, even though they do not portray obvious symptoms of inattention and hyperactivity-impulsivity. These vulnerabilities may translate into difficulties at school, but may be easily overlooked in the absence of clear ADHD symptoms. It may prove helpful to (some) siblings of affected participants to be neuropsychologically examined themselves in order to obtain a cognitive profile with weaknesses and strengths.

Increasing knowledge about the etiology of ADHD will eventually lead to earlier detection of (precursors of) the disorder, more reliable methods of diagnosis and, hopefully, to better treatments. In the (near) future, it may be envisaged that the structure of the DSM will change and incorporate knowledge about the etiology of ADHD. A view of the axes of a future DSM may look something like this (based on a talk by Prof dr. J. Hudziak) (Table 16.1).
Table 16.1 View of the axes of a future DSM.

**Axis I: Genotype**
Genes related to diseases, symptoms, resiliency, and drug response. This may also include registering a possible generalized enhanced genetic risk if first-degree relatives suffer from certain disorders.

**Axis II: Neurobiological and neuropsychological endophenotypes**
Structural and functional neurological measures, neurochemical measures, neuropsychological measures, neuropsychological profile.

**Axis III: Behavioral phenotype**
Expression of disease-related behaviors, including their range, frequency, and severity.

**Axis IV: Environmental factors**
Environmental factors that may moderate or mediate the relationship between genotype and endophenotypes, on the one hand, and behavioral phenotype, on the other hand.

**Axis V: Therapeutic targets and means of intervention**
Defining at which axis what type of intervention will focus