Putative genetic and environmental factors influencing Attention-Deficit/Hyperactivity Disorder (ADHD) in a South African sample

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List of abbreviations

(CA)n – Dinucleotide repeat sequence
11β-HSD - 11β-hydroxysteroid dehydrogenase
5-HTTLPR – Serotonin-transporter-linked-polymorphic region
ACTH – Adrenocorticotropic hormone
ADHASA - Attention Deficit and Hyperactivity Support Group of Southern Africa
ADHD - Attention-Deficit/Hyperactivity Disorder
ADRA2A - Alpha-2A adrenergic receptor gene
ANCOVA - Analysis of covariance
ANOVA - Analysis of variance
APA - American Psychiatric Association
ASD - Autism Spectrum Disorders
ASRS - ADHD Self-Report Scale
CD - Conduct Disorder
CDCV – Common disease/common variant hypothesis
CDRV – Common disease/rare variant hypothesis
CNV - Copy number variant
COMT – Catachol-O-methyltransferase
COMT - Catachol-O-methyltransferase gene
CP - Conduct problems
CRH – Corticotropin-releasing hormone
CRH – Corticotropin-releasing hormone gene
DAT – Dopamine transporter
DAT1 - Dopamine transporter gene
DAT1 3’ uVNTR – Variable number of tandem repeats polymorphism in the 3’ untranslated region of the dopamine transporter gene
DNA – Deoxyribonucleic acid
DRD2 – Dopamine D2 receptor
DRD2 – Dopamine D2 receptor gene
*DRD2* Taq1A – Single nucleotide polymorphism in the 3’ untranslated region of the *DRD2* gene
DRD4 – Dopamine D4 receptor
DRD4 – Dopamine D4 receptor gene
*DRD4* exon III VNTR – Variable number of tandem repeats polymorphism in exon three of the dopamine D4 receptor gene
DRD5 – Dopamine D5 receptor
*DRD5* – Dopamine D5 receptor gene
*DRD5* (CA)n – Microsatellite polymorphism in the *DRD5* gene
DSM - Diagnostic and Statistical Manual of Mental Disorders
DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DSM-III – Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition
DSM-IV - Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
*DBH* – Dopamine Beta-hydroxylase gene
DBH – Dopamine-Beta-hydroxylase
GXEs - Gene-environment interactions
HRAS – Harvey Ras oncogene
*LPHN3* – Latrophilin 3 gene
MAOA – Monoamine oxidase A
MAOA – Monoamine oxidase A gene
*MAOA-uVNTR* – Monoamine oxidase A upstream variable number of tandem repeats polymorphism
MDM - Mean difference model
MPH – Methylphenidate
ND – Nicotine dependence
ODD - Oppositional Defiant Disorder
PCR - Polymerase chain reaction
PET - Positron-emission tomography
PMT - Polygenic multiple threshold model
RRR – Relative risk ratio
SD – Standard deviation
SDS – Sodium dodecyl sulfate
SHR - Spontaneously hypertensive rat
SLC6A4 – Serotonin transporter gene
SNAP-IV - Swanson, Nolan, and Pelham IV Questionnaire
SNP - Single nucleotide polymorphism
SPSS – Statistical Package for the Social Sciences
STATA - Data Analysis and Statistical Software for Professionals
UTR - Untranslated region
UV - Ultraviolet
VNTR - Variable number of tandem repeats
WHO - World Health Organisation
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Chapter 1

Motivation and Overview

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Attention deficit/hyperactivity disorder (ADHD) is defined as “a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development” (5th ed.; DSM–5; American Psychiatric Association [APA], 2013). For a diagnosis to be made, the onset of symptoms need to occur before the age of 12 years. Children diagnosed with ADHD may display symptoms of predominantly inattention, predominantly hyperactivity-impulsivity, or both inattention and hyperactivity-impulsivity. In accordance, three distinct presentations of the disorder are specified in the newest version of the DSM-5, namely predominantly inattentive presentation, predominantly hyperactive-impulsive presentation, and combined presentation (APA, 2013).

The estimated world-wide prevalence of ADHD is around 5% (Polanczyk, De Lima, Horta, Biederman, & Rohde, 2007), making this disorder one of the most frequently diagnosed childhood psychiatric disorders (Mill, & Petronis, 2008). ADHD has a significant effect on the life of not only the diagnosed child, but also the child’s family (Harpin, 2005). Studies have shown greater family dysfunction in families with a child with ADHD, manifesting as severe marital discord, lower levels of cohesiveness and organization, and more conflict (Foley, 2011; Pheula, Rohde, & Schmitz, 2011). Lower parent reported family quality of life, less parental warmth, less consistent parenting and more hostile parenting styles, have also been reported (Cussen, Sciberras, Ukoumunne, & Efron, 2012).

Concerning the effects on the child diagnosed with ADHD, a review by Loe, and Feldman (2007) noted a number of adverse effects of the disorder on academic and educational outcomes. These included poor grades, low reading and maths standardized test scores, and increased rates of repeating grades. Children with ADHD are also placed in detention more frequently, are expelled more frequently, and show relatively low rates of high school graduation and post-secondary education (Loe, & Feldman, 2007). Apart from academic and educational outcomes, ADHD also affects children’s relationships with their peers (Hoza, 2007; Hoza, Mrug, Gerdes, Hinshaw, Bukowski, Gold, et al., 2005; Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012). Children with ADHD have been shown to have higher rates of rejection by their peers (Hoza, Mrug, Gerdes, Hinshaw, Bukowski, Gold, et al., 2005; Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012). They also tend to be less well liked by their peers, and have fewer dyadic friends than children not diagnosed with ADHD (Hoza, Mrug, Gerdes, Hinshaw, Bukowski, Gold, et al., 2005). Peer relationships
provide a context where children can interact socially with others of equal status, and as such constitute an important developmental context for children. Through these relationships, children learn skills such as cooperation, negotiation and conflict resolution. They acquire new behaviours, attitudes and experiences that influence them for the rest of their lives (Hoza, 2007; Rubin, Bukowski, & Parker, 2007). It is thus not surprising that the peer rejection experienced by children with ADHD has been linked to negative long-term outcomes such as cigarette smoking, delinquency, anxiety and global impairment (Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012).

Studies have found that the symptoms of ADHD also frequently persist into adulthood (Klein, Mannuzza, Olazagasti, Roizen, Hutchison, Lashua, et al., 2012). In a review of the literature, Rösler, Casas, Konofal, and Buitelaar (2010) concluded that symptoms of ADHD in adulthood significantly impacted on an individual’s work life, social life and relationships. In addition, ADHD is often accompanied by co-morbid disorders, in particular Oppositional Defiant Disorder (ODD), with up to 60% of children diagnosed with ADHD also receiving a diagnoses of ODD (Biederman, 2005; Connor, Steeber, & McBurnett, 2010; Cuffe, Visser, Holbrook, Danielson, Geryk, Wolraich, et al., 2015; Inci, Ipci, Akyol Ardıç, & Ercan, 2016; Joelsson, Chudal, Gyllenberg, Kesti, Hinkka-Yli-Salomäki, Virtanen, et al., 2016). This high rate of co-morbidity with ODD adds to the morbidity of the disorder by increasing the chance of negative outcomes (Connor, & Doerfler, 2008; Connor, Steeber, & McBurnett, 2010; Dalsgaard, Mortensen, Frydenberg, & Thomsen, 2002; Waschbusch, 2002).

It should thus be clear that the negative consequences of ADHD are many and varied, not only impacting individuals throughout their lives, but also their family members and loved ones. Stimulant medication, in the form of methylphenidate or amphetamines, has been proven as an effective treatment for children with ADHD, with an approximate response rate of 70% (Greenhill, Swanson, Vitiello, Davies, Clevenger, Wu, et al., 2001; Gunter, 2013). Whilst impressive, that still means that stimulant medication does not improve ADHD symptoms in roughly 30% of children. In addition, numerous side effects have been reported for stimulant medications, including insomnia, decreased appetite, headaches, stomach aches, and a dull/listless appearance (Greenhill, Swanson, Vitiello, Davies, Clevenger, Wu, et al., 2001; Lee, Grizenko, Bhat, Sengupta, Polotskaia, & Joober, 2011). There is thus still room for improvement regarding pharmacotherapy for ADHD.
Understanding the aetiology of the disorder is a crucial step in the discovery of better treatment strategies. Great advances have been made through both behavioural genetic (i.e. family, twin and adoption studies) and molecular genetic studies in this regard (e.g. Smalley, McGough, Del’Homme, NewDelman, Gordon, Kim, et al., 2000; Gizer, Ficks, & Waldman, 2009; Faraone, & Mick, 2010; Nikolas, & Burt, 2010). In addition, environmental factors influencing the disorder have been found to mostly be associated with factors surrounding pregnancy and birth (Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al., 2012). Concerning behavioural genetic studies, ADHD has been found to be one of the most heritable disorders of childhood, with heritability estimates in the region of 70% (Nikolas, & Burt, 2010). A large number of candidate gene studies have also been conducted on the disorder, with associations found especially with genes in the dopaminergic neurotransmitter system (e.g. Li, Sham, Owen, & He, 2006; Gizer, Ficks, & Waldman, 2009; Faraone, & Mick, 2010; Wu, Xiao, Sun, Zou, & Zhu, 2012). That said, molecular genetic studies of ADHD have been plagued by conflicting findings, with no specific gene consistently found to be associated with the disorder (Gizer, Ficks, & Waldman, 2009; Li, Chang, Zhang, Gao, & Wang, 2014; Sun, Yuan, Shen, Xiong, & Wu, 2013). There is abundant evidence that ADHD is an aetiologically heterogeneous disorder, with aetiological mechanisms differing for different subgroups of patients (Crosbie, & Schachar, 2001; Nigg, Willcutt, Doyle, & Sonuga-Barke, 2005; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015). The conflicting findings reported thus far may be the result of study populations being heterogeneous for these aetiologically distinct subgroups (Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar et al., 2014; Virkud, Todd, Abbacchi, Zhang, & Constantino, 2009). Furthermore, the conflicting findings may also be due to factors in the environment interacting with genetic factors to influence the disorder (Faraone, & Mick, 2010; Gizer, Ficks, & Waldman, 2009).

This study will aim to explore the validity of possible explanations for the conflicting findings by making use of genetically informative study designs. This will aid future researchers to explore more successfully the aetiology of the disorder by selecting more homogeneous sample populations, and by including possible moderating variables into their study designs. In addition, it is noteworthy that neither behavioural genetic nor molecular genetic studies into the aetiology of ADHD have ever been conducted in a sample from South
Africa. Consequently, there is a dearth of knowledge regarding both the heritability and the molecular genetics of ADHD in the South African population. Through the exploration of reasons for the conflicting findings by making use of genetically informative study designs, this study will also begin to address this shortcoming, and thus serve as a starting point for future research into the aetiology of ADHD in South Africa.

It should however be noted that the aim of the study is not to obtain a sample that is representative of the South African population, since neither the time nor the financial resources were available for this level of recruitment. Rather, the study focuses on beginning the process of exploring genetic factors influencing ADHD in South Africa and exploring methods that can be used to investigate genetic factors in a country with limited resources for large-scale molecular genetic studies. This study will thus aim to serve as a starting point for future research into this topic. Generalizations from the sample to the population will therefore not be made. Previous behavioural genetic studies that have been published in peer-reviewed journals also utilised samples that were not representative of the population (e.g. Agudelo, Gálvez, Fonseca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Van Dyk, Springer, Kidd, Steyn, Solomons, & Van Toorn, 2014), and thus this was not viewed as a factor that would prevent publication.

In addition, as was done in a previously published behavioural genetic study in South Africa (Van Dyk, Springer, Kidd, Steyn, Solomons, & Van Toorn, 2014), race/ethnicity was not emphasised in this study. The decision not to emphasise ethnicity is in line with strong arguments against subdividing a sample based on ethnicity in genetic research. In essence, making use of racial/ethnic categories in genetic research may lead to stereotyping racial and ethnic groups as being clearly delineated, and associating certain health outcomes with all individuals in a specific group, rather than only with individuals who show the disease/disorder (Race, Ethnicity, and Genetics Working Group, 2005; Sankar, Cho, Condit, Hunt, Koenig, Marshall, et al., 2004). In addition, although there is no evidence from genetic research showing that one racial/ethnic group is superior to another, some individuals still distort genetic findings to serve their prejudiced outlooks (Race, Ethnicity, and Genetics Working Group, 2005).
Furthermore, it should be noted that, as was done in previous studies published in peer-reviewed journals (e.g. Crosbie, Arnold, Paterson, Swanson, Dupuis, Li, et al., 2013; Diamantopoulou, Henricsson, & Rydell, 2005; Ronald, Simonoff, Kuntsi, Asherson, & Plomin, 2008), in all chapters in this thesis a community sample rather than a clinical sample was utilised. For the subset of the sample for who molecular genetic analysis were conducted, participants’ ADHD diagnostic status as reported by their parents was confirmed directly by the diagnosing healthcare professionals. However, for the remainder of the sample, classification of participants as “diagnosed with ADHD” or “not diagnosed with ADHD” was derived from self-report of diagnosis by a healthcare professional (for parents) or by parent-report of diagnosis by a healthcare professional (for children). Although the researcher acknowledges that a structured diagnostic interview by a healthcare professional for the full sample would have been preferable, the decision was made through consultation with the supervisors to rather use self- or parent-report of diagnosis by a healthcare professional due to the considerable cost and time involved in healthcare professionals conducting structured diagnostic interviews. This decision was, however, only taken after careful scrutiny of studies published in peer-reviewed scientific journals to ensure that studies using this form of diagnostic classification are indeed published. Please see LeFever, Villers, and Morrow (2002); Lesesne, Visser, and White (2003); Braun, Kahn, Froehlich, Auinger, and Lanphear (2006); Larson, Russ, Kahn, and Halfon (2011); and Visser, Danielson, Bitsko, Holbrook, Kogan, Ghandour, et al. (2014) as examples of where this methodology was employed and the papers subsequently published in peer-reviewed journals. This decision was further supported by a study conducted by Visser, Danielson, Bitsko, Perou, and Blumberg (2013) in which the researchers compared prevalence rates of ADHD as deduced from parent-report of diagnosis by a healthcare professional with that of documented ADHD diagnosis in medical records in the same geographical area and found that the prevalence rates were statistically indistinguishable.

Through making use of a family study design, this study will firstly attempt to determine whether the symptom dimensions of ADHD do indeed run in families. This will provide evidence for genetic factors influencing the disorder, a crucial condition that needs to be met prior to conducting further behavioural genetic analysis into the aetiology of the disorder. Next, by making use of a family study design, this study will explore the aetiologically
heterogenous nature of the disorder, through trying to determine whether causal factors differ for different subgroups of patients. More specifically, this study will explore whether ADHD combined type and ADHD predominantly inattentive type are varying presentations of the same disorder, or distinct disorders; the aetiological nature of ADHD comorbid with ODD; the aetiological basis for the gender differences found in ADHD; the distinction between simplex and multiplex ADHD; and whether rare genes influence the disorder for a subgroup of patients. In addition, possible interaction effects between genes and environmental factors, possibly resulting in conflicting findings, will be explored. Consequently, this study will aim to answer the following research questions:

➢ Is there familial aggregation of ADHD symptoms in families in a South African sample, and can it thus be viewed as a heritable disorder?
➢ Are ADHD combined type and ADHD inattentive type distinct disorders, or varying presentations of the same disorder?
➢ What is the aetiological nature of the co-occurrence of ADHD and ODD in a sample from South Africa?
➢ What is the aetiological nature of the gender differences observed for ADHD in a sample from South Africa?
➢ What is the aetiological nature of simplex versus multiplex ADHD in a sample from South Africa?
➢ Do rare genetic variants play a role in ADHD for some patients in a sample from South Africa?
➢ Are there any significant interaction effects between genes and environmental factors in the aetiology of ADHD in a sample from South Africa?

This thesis is written in the form of a series of scientific articles, each one dealing with a certain aspect of the study. Although the names of the promoter and co-promoters are listed as authors for the scientific papers, the research was conducted by Nadia Fouché and she planned and wrote the thesis. Due to the article format, duplication of short sections (for example materials and methods) were unavoidable. Ethical approval of this study was gained through the Health Research Ethics Committee of the University of the Free State (REC Reference Nr: 230408-011; ECUFS Nr: 67/2015) (Appendix F).
This chapter is followed by a comprehensive review of the relevant literature. The literature review chapter is followed by a short introduction chapter in which the use of the original sample in each of the subsequent chapters is explained to provide context and clarify why the number of participants differ in each chapter. Thereafter, the research chapters follow, starting with a study into the familial aggregation of ADHD subtypes in a South African sample. This is followed by four chapters investigating the possibility that aetiologically distinct subgroups exist within the ADHD population. In the first chapter to do so, the question is investigated whether ADHD combined type and predominantly inattentive type are distinct disorders, or varying presentations of the same disorder. In the second chapter the aetiology of the comorbidity of ADHD and ODD is investigated, whilst the third chapter presents possible explanations for the well documented differences in ADHD prevalence between the sexes. In the fourth chapter the possible distinction between simplex and multiplex ADHD was determined through investigating whether pregnancy and delivery complications influence these two forms of the disorder differently. Hereafter, two chapters follow in which the molecular genetic architecture of ADHD is investigated. In the first chapter, the presence of a possible interaction effect between the \textit{DAT1 3’ uVNTR} polymorphism and pregnancy and/or delivery complications is examined. In the second chapter, and final research chapter of the thesis, the presence of rare sequence variations within the \textit{MAOA-uVNTR} is tested for in a sample of children diagnosed with ADHD in South Africa.

This chapter is followed by an overall discussion and conclusion chapter where all the results of the different articles are discussed. This is followed by a summary of the thesis in both English and Afrikaans, and a chapter containing all the references used in the thesis. Finally, all the raw data and extracts of the statistical analyses are attached as Appendices.
Chapter 2

Literature review

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2.1 Introduction

According to the latest edition (5th ed.) of the Diagnostic and Statistical Manual of Mental Disorders (5th ed.; DSM-5; American Psychiatric Association [APA], 2013) Attention-Deficit/Hyperactivity Disorder (ADHD) has at its core, symptoms of inattention and/or hyperactivity-impulsivity. These symptoms need to be persistent (had to have occurred for at least six months), have their origin before the age of 12 years, and must markedly interfere with functioning or development. The DSM-5 stipulates that the symptoms of inattention in ADHD manifest as wandering off task, not being able to persist in anything, lacking focus, and being disorganised. Symptoms of hyperactivity include inappropriate and excessive motor activity, fidgeting, tapping, or talking. Finally, impulsivity manifests as hasty actions without adequate forethought and with a high potential for harm to the individual. This may include a desire for immediate rewards, and behaviours such as social intrusiveness (APA, 2013).

The DSM-5 further classifies the disorder into three subtypes, namely:

- **Combined subtype** – diagnosed if both inattention and hyperactivity-impulsivity symptoms are present for at least six months;
- **Predominantly inattentive subtype** – diagnosed if only an adequate number of inattention symptoms, but not sufficient hyperactive-impulsive symptoms, are present for at least six months;
- **Predominantly hyperactive-impulsive subtype** – diagnosed if only an adequate number of hyperactivity-impulsivity symptoms, but not inattention symptoms, are present for at least six months (Diagnostic and Statistical Manual of Mental Disorders, 2013).

Numerous detrimental consequences have been linked to a diagnosis of ADHD, not only in the diagnosed individual, but also in their families (Harpin, 2005). Academic and school difficulties associated with the disorder include children achieving lower grades, more frequently repeating grades, more frequently receiving some sort of punishment, and more frequently being expelled. In addition, children with ADHD show relatively low high school graduation and low tertiary education rates (Loe, & Feldman, 2007). In addition, behavioural problems with peers are also frequently reported, with diagnosed children frequently facing
peer rejection. They also tend to have fewer dyadic friends than unaffected children (Hoza, 2007; Hoza, Mrug, Gerdes, Hinshaw, Bukowski, Gold, et al., 2005; Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012). Peer interaction, in turn, plays a crucial role in child development (Hoza, 2007; Rubin, Bukowski, & Parker, 2007). It is, therefore, not surprising that the peer rejection frequently seen in children diagnosed with ADHD, leads to negative long-term consequences such as cigarette smoking, delinquency, anxiety, and global impairment (Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012).

Apart from the negative consequences on the diagnosed individual, families of children diagnosed with ADHD also face unique challenges. Greater family dysfunction is frequently experienced, manifesting as severe marital discord, lower levels of cohesiveness and organisation, and more conflict. In addition, less parental warmth, less consistent parenting, and a more hostile parenting style, are frequently reported in families of children diagnosed with ADHD (Cussen, Sciberras, Ukoumunne, & Efron, 2012; Foley, 2011; Pheula, Rohde, & Schmitz, 2011). Given these negative ramifications, effective treatment strategies are crucial to lessen the impact on affected individuals and their families. Great strides have been made in this regard, with effective pharmaceutical (Greenhill, Swanson, Vitiello, Davies, Clevenger, Wu, et al., 2001; Gunter, 2013) and behavioural (Daley, Van Der Oord, Ferrin, Danckaerts, Doepfner, Cortese, et al., 2014) treatment strategies available. However, given that pharmaceutical treatment has a proven positive response rate of only 70% (Greenhill, Swanson, Vitiello, Davies, Clevenger, Wu, et al., 2001; Gunter, 2013), there is still room for improvement.

Before treatment strategies can be improved upon, knowledge of the aetiology of a disorder is crucial. As will be shown in this review, studies into the aetiology of ADHD have thus far been plagued by many conflicting findings. Thus, the aim of this study is to put forth, and test, possible reasons for the high rate of conflicting findings, with the ultimate aim of furnishing future researchers with methodological tools to better be able to come to definitive conclusions regarding the aetiology of ADHD.
2.2 Prevalence of ADHD

Five percent of children in most cultures will meet the diagnostic criteria for ADHD (APA, 2013). A meta-analysis by Polanczyk, De Lima, Horta, Biederman, and Rohde (2007) analysed data from 102 studies from regions all around the world. This meta-analysis found a worldwide prevalence of ADHD of 5.29%. A review on the prevalence of ADHD in African countries concluded that rates of ADHD varied from 5.4% to 8.7% amongst school children in various African countries (Bakare, 2012). This review cautioned that, due to the limited number of publications of this nature in Africa, more studies are needed for conclusive figures. With particular reference to the South African context, Meyer, Eilertsen, Sundet, Tshifularo, and Sagvolden (2004) found a prevalence of 5.5% for ADHD in a sample of 6 094 South African primary school children. More recent data is available on the prevalence of adult ADHD patients presenting to psychiatric practices, with Schoeman, De Klerk, and Kidd (2015) noting an estimated prevalence of 10% (for older adults) to 22% (for young adults).

Concerning the prevalence of ADHD subtypes, Skounti, Philalithis, and Galanakis (2006) summarised results from studies on the prevalence of ADHD from 1995 to 2004. They concluded that ADHD, predominantly inattentive type, occurred most frequently, followed by ADHD combined type and then ADHD hyperactive-impulsive type. These results were confirmed in a worldwide meta-analysis by Willcutt (2012), and in South Africa, by Meyer, Eilertsen, Sundet, Tshifularo, and Sagvolden (2004).

2.3 Gender and ADHD

One striking feature concerning the prevalence of ADHD is that it is more prevalent in boys than in girls worldwide (APA, 2013; Meyer, Eilertsen, Sundet, Tshifularo, & Sagvolden, 2004; Ramtekkar, Reiersen, Todorov, & Todd, 2010; Willcutt, 2012). A meta-analysis showed that according to parent ratings, the boy-to-girl ratio for the ADHD combined type was 2.4:1; for the inattentive type 2.0:1, and for the hyperactive-impulsive type 2.6:1 (Willcutt, 2012).

Hasson, and Fine (2012) noted that the influence of gender on ADHD is not clearly understood. What is clear, however, is that ADHD does occur in both males and females (Willcutt, 2012). Several similarities between male and female ADHD have been noted. In particular, the core symptoms of ADHD occur in both males and females. Both males and
females are also at an increased risk for comorbid psychiatric disorders, as well as cognitive impairment, psychosocial impairment, and impaired family functioning (Biederman, Faraone, Mick, Williamson, Wilens, Spencer, et al., 1999; Biederman, Mick, Faraone, Braaten, Doyle, Spencer, et al., 2002; Biederman, Kwon, Aleardi, Chouinard, Marino, Cole, et al., 2005; Biederman, Faraone, Monuteaux, Bober, & Cadogen, 2004). Furthermore, a study by DuPaul, Jitendra, Tresco, Junod, Volpe, and Lutz (2006) showed that children diagnosed with ADHD showed impaired school functioning across multiple domains, regardless of gender.

Apart from these similarities, many studies have also shown important gender differences in ADHD, especially on the prevalence rates (Ramtekkar, Reiersen, Todorov, & Todd, 2010; Willcutt, 2012). In addition, a meta-analysis by Gershon, and Gershon (2002) showed that boys with ADHD had higher rates of hyperactivity, inattention, impulsivity, and externalising problems than girls with ADHD. In contrast, girls diagnosed with ADHD tended to have greater intellectual impairments and more internalising problems than boys. This meta-analysis mostly replicated the findings from a previous one performed by Gaub, and Carlson (1997), with the exception that latter authors found lower levels of internalising problems in girls from a non-referred sample compared to boys. More recently, Skogli, Teicher, Andersen, Hovik, and Øie (2013) pointed out higher rates of self-reported anxiety in females than in males with ADHD.

Two closely related models proposed to explain the gender differences observed in ADHD are the polygenic multiple threshold model (PMT) (Rhee, & Waldman, 2004; Rhee, Waldman, Hay, & Levy, 1999) and the mean difference model (MDM) (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015). Multifactorial disorders like ADHD are assumed to be caused by multiple genetic and environmental factors. These genetic and environmental risk factors combine additively, and the PMT model suggests that the disorder will manifest in individuals in which these risk factors exceed a certain threshold. Thus, in individuals who do not have ADHD, the particular combination of risk factors does not exceed this critical level of liability. In addition, the PMT model posits that multiple thresholds exist for different groups in a population, and in the case of ADHD, between males and females (Rhee, & Waldman, 2004; Rhee, Waldman, Hay, & Levy, 1999). If the PMT model holds true, it would be expected that relatives of female probands would be more likely to have ADHD than relatives of male probands. This is because females will require and transmit a higher liability
for the disorder, making relatives of female probands more likely to have the disorder (Rhee, Waldman, Hay, & Levy, 1999). In addition, relatives of girls with ADHD would be expected to have a greater number of, and more severe symptoms than, relatives of boys with ADHD (Smalley, McGough, Del’Homme, NewDelman, Gordon, Kim, et al., 2000). This same effect has been found for idiopathic clubfoot, which is twice as common in males as in females, and is known as the Carter effect. As is suggested here to be the case with ADHD, research has found that females require a greater genetic load to be affected with clubfoot than males, resulting in children of affected mothers being more likely to be affected than children of affected fathers (Kruse, Dobbs, & Gurnett, 2008).

Similar to the PMT model, the MDM model posits that the distribution of liability for females is shifted in the less-affected direction compared to that of males. Stated differently, males have a shifted distribution compared to that of females, with the mean for the males closer to the diagnostic threshold. Thus, if the variances in distribution of liability for the male and female populations are equal, more males will fall into the affected tail of the distribution, and will more frequently be diagnosed with ADHD (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015). Should the MDM hold true, females diagnosed with ADHD should carry no greater liability for the disorder than males diagnosed with ADHD, and thus the relatives of diagnosed females should not display greater ADHD symptom severity than the relatives of diagnosed males. Since, according to the MDM, males in the population carry a greater liability for the disorder than females, males would be expected to have more severe ADHD scores than females (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015).

Various studies have been conducted on the effect of parental gender on ADHD symptoms in offspring, with contradictory results. Some studies have provided support for the PMT model by showing that children with a maternal history of ADHD show higher ADHD symptom severity than children with a paternal history of ADHD (Goos, Ezzatian, & Schachar, 2007; Agha, Zammit, Thapar, & Langley, 2013). Also in line with the PMT model, in a multiplex family study of ADHD, Smalley, McGough, Del’Homme, NewDelman, Gordon, Kim, et al. (2000) found that the rate of ADHD in parents was higher in families in which at least one girl had the disorder, compared to families where only boys were affected. In contrast, Biederman, Mick, Faraone, Braaten, Doyle, Spencer, et al. (2002) found no significant difference in the ADHD clinical manifestation in children with mothers with ADHD compared
to children with fathers with ADHD. This finding provided evidence against the validity of the PMT model. Confounding the issue even further is a finding by Takeda, Stotesbery, Power, Ambrosini, Berrettini, Hakonarson, et al. (2010) which indicated that paternal ADHD severity, instead of maternal ADHD severity, was significantly associated with ADHD severity in children with ADHD. Further studies are clearly needed to clarify whether the PMT model is a valid explanation for the gender differences observed in ADHD.

Another likely cause of the male-to-female prevalence bias observed in ADHD might be the different sex-chromosome constitution of males and females. Males inherit one X-chromosome (always from the mother) and one Y-chromosome. Females on the other hand, inherit two X-chromosomes (one from the mother and one from the father). The result is that the expression of certain gene products will be higher in the female brain than in the male brain, since up to 20% of genes on the X-chromosome escape X-inactivation (Lyon, 1999; Reik, & Lewis, 2005; Trent, & Davies, 2012). In addition, the expression of alleles on the single male X-chromosome can have a direct influence on the phenotype. This is due to the absence of a second allele on a corresponding X-chromosome that can mask the influence of this allele, as is the case in females (Trent, & Davies, 2012). This has been found to be the case for diseases such as haemophilia A, a bleeding disorder resulting from a deficiency of the factor VIII blood clotting protein, and predominantly present in males. Haemophilia A shows X-linked recessive inheritance, with the locus located near the distal end of the long arm of the X chromosome. Due to females having two X-chromosomes, factor VIII levels have been found to be half-normal in carrier females, resulting in a reduced clinical phenotype compared to males (Turnpenny, & Ellard, 2016). If, like for haemophilia, the differential sex chromosome constitution between males and females is shown to be the cause of the differences observed between the sexes for ADHD, this implies genes on the X-chromosome play a role in ADHD.

However, before a search for specific genes are initiated, it is important to determine the magnitude of the genetic influence on ADHD, as is done by family, twin, and adoption studies.
2.4 Heritability of ADHD

There is continued evidence from family, twin, and adoption studies that ADHD is a highly heritable disorder (Biederman, 2005; Faraone, & Doyle, 2001; Lichtenstein, Carlström, Råstam, Gillberg, & Anckarsäter, 2010; Nikolas, & Burt, 2010; Sprich, Biederman, Crawford, Mundy, & Faraone, 2000; Takeda, Stotesbery, Power, Ambrosini, Berrettini, Hakonarson, et al., 2010; Thapar, Cooper, Jefferies, & Stergiakouli, 2012). A twin study by Lichtenstein, Carlström, Råstam, Gillberg, and Anckarsäter (2010) in a population of 16 858 Swedish twins found heritability estimates for ADHD as high as 79%. Furthermore, Nikolas, and Burt (2010) carried out a meta-analysis to determine the magnitude of genetic and environmental influences on ADHD symptom dimensions. Results showed that genetic factors accounted for 71% of the variance in symptoms of inattention and for 73% of the variance in symptoms of hyperactivity-impulsivity (Nikolas, & Burt, 2010).

Apart from the overall estimates of genetic factors influencing ADHD mentioned previously, studies have also looked at the type of genetic factors influencing the disorder and its dimensions (Nikolas, & Burt, 2010). Through adoption and twin studies, researchers separate the variance in behaviours into four components (Burt, 2009; Nikolas, & Burt, 2010). Additive genetic variance represents that part of the variance in behaviour that is explained by the cumulative effects of individual genes. Dominant genetic variance (also called non-additive genetic variance) represents that part of the variance explained by the interaction between alleles. It is important to note that only additive genetic effects will result in similarities between first-degree relatives for a trait. The shared environmental component represents that part of the environment that is shared by members of a family, and thus serves to make the family members more similar in a trait. Finally, the non-shared environment represents the environmental factors not shared by members of a family and serves to make family members dissimilar from each other (Burt, 2009; Nikolas, & Burt, 2010; Plomin, & Daniels, 2011).

Concerning the symptom dimensions of hyperactivity-impulsivity and inattention, the meta-analysis by Nikolas, and Burt (2010) on twin and adoption studies indicated that additive genetic factors have a greater influence on hyperactivity-impulsivity than inattention symptom dimensions. Non-additive genetic influences played a greater role in
inattention than hyperactivity-impulsivity. The influence of the shared environment was found to be negligible (Nikolas, & Burt, 2010). These findings are in contrast to findings from studies examining familial clustering of ADHD symptoms. Using quantitative measures of ADHD subtypes, correlations between first-degree relatives (sibling-pairs) were higher for the inattention than for the hyperactive-impulsive symptom dimension (Smalley, McGough, Del'Homme, NewDelman, Gordon, Kim, et al., 2000). Similarly, in a more recent study, it was found that parental ADHD had a greater effect on inattentive than hyperactive-impulsive symptoms (Takeda, Stotesbery, Power, Ambrosini, Berrettini, Hakonarson, et al., 2010). This would suggest a greater influence of either the shared environment or additive-genetic factors on inattention than hyperactivity-impulsivity.

From the literature, it thus appears that the magnitude of the genetic influences on ADHD has been well established. It is noteworthy, however, that apart from a recently published study which took into account the effect of maternal ADHD (Van Dyk, Springer, Kidd, Steyn, Solomons, & Toorn, 2014), no family, twin or adoption studies of this kind have been carried out in the South African population. Consequently, there is a great dearth of knowledge regarding the variable influence of genetic and environmental factors on ADHD in the African continent. There is significant heterogeneity in the underlying genetic factors causing ADHD in different world populations (Ogdie, Bakker, Fisher, Francks, Yang, Cantor, et al., 2005; Zhou, Dempfle, Arcos-Burgos, Bakker, Banaschewski, Biederman, et al., 2008). Findings from studies in other populations can, thus, not necessarily be generalised to the South African context. It is nonsensical to perform molecular genetic studies on a disorder that is not influenced by genetic factors. Therefore, it is pertinent that studies first examine the magnitude of the genetic influence on ADHD in Africa before attempting to conduct molecular genetic analysis. The current study will be the first study of this kind in South Africa, making use of a family study design.

2.5 Molecular genetics of ADHD

2.5.1 Dopamine and ADHD

One of the theories of ADHD that has stood the test of time is the dopamine deficit theory (Spencer, Biederman, Madras, Faraone, Dougherty, Bonab, et al., 2005; Swanson, Kinsbourne, Nigg, Lanphear, Stefanatos, Volkow, et al., 2007; Tarver, Daley, & Sayal, 2014).
Various lines of evidence exist that support the important role of dopamine in the development of ADHD. Already in 1971, Wender proposed that the range of symptoms seen in ADHD might be because of subtle abnormalities in the dopamine neurotransmitter systems (Wender, 1973). More than three decades later, Swanson, Kinsbourne, Nigg, Lanphear, Stefanatos, Volkow, et al. (2007) summarised the accumulated evidence to support Wender’s claim. This summary indicates that brain imaging consistently shows that the caudate nucleus and globus pallidus are smaller in ADHD than in healthy individuals. These brain areas contain a high density of dopamine receptors (Swanson, Kinsbourne, Nigg, Lanphear, Stefanatos, Volkow, et al., 2007).

A second line of evidence comes from the effects of the drug methylphenidate (MPH), one of the most frequently prescribed, and most effective treatments for ADHD in children and adults (Gizer, Ficks, & Waldman, 2009; Hanwell, Senanayake, & De Silva, 2011; Maia, Cortese, Caye, Deakin, G.V. Polanczyk, C.A. Polanczyk, et al., 2017; Volkow, Wang, Fowler, Gatley, Logan, Ding, et al., 1998). MPH works by blocking dopaminergic transporters in the human brain. Positron-emission tomography (PET) studies have shown that, at the therapeutic dose, MPH blocks up to 70% of brain dopaminergic transporters (Volkow, Wang, Fowler, Gatley, Logan, Ding, et al., 1998; Zimmer, 2017). This results in what is considered MPH’s main mechanism of therapeutic action, namely an increase in the extracellular concentration of dopamine in the human brain (Gizer, Ficks, & Waldman, 2009; R.C. Spencer, Devilbiss, & Berridge, 2015; Volkow, Wang, Fowler, Gatley, Logan, Ding, et al., 1998, Volkow, Wang, Fowler, Logan, Gerasimov, Maynard, et al., 2001). The efficiency of MPH in reducing symptoms of ADHD through an increase in extracellular dopamine provides direct evidence of the important role that this neurotransmitter plays in ADHD.

Finally, animal models of ADHD have repeatedly shown functional impairment of the dopaminergic system (Bock, Breuer, Poeggel, & Braun, 2017; Russell, Sagvolden, & Johansen, 2005; Sontag, Tucha, Walitza, & Lange, 2010). In particular, the spontaneously hypertensive rat (SHR) is considered to be one of the most appropriate animal models for ADHD (Dela Peña, & Cheong, 2013; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). SHR rats have been shown to have disturbed uptake, storage and metabolism of dopamine (Russell, 2002, 2003; Sontag, Tucha, Walitza, & Lange, 2010).
Given this plethora of evidence, it is not surprising that the genes affecting dopaminergic neurotransmission are seen as crucial study elements in the search for the causes of ADHD (Gizer, Ficks, & Waldman, 2009; Kirley, Hawi, Daly, McCarron, Mullins, Millar, et al., 2002; Li, Sham, Owen, & He, 2006; Maher, Marazita, Ferrell, & Vanyukov, 2002; Wu, Xiao, Sun, Zou, & Zhu, 2012).

2.5.2 Dopaminergic genes as candidate genes for ADHD

2.5.2.1 The Dopamine transporter gene (DAT 1)

Vandenbergh, Persico, Hawkins, Griffin, Li, Jabs, et al. (1992) mapped the dopamine transporter gene (DAT1) to the short arm of chromosome 5 (5p15). This gene has been shown to code for a protein known as the dopamine transporter (DAT) protein. This protein is responsible for the reuptake of the neurotransmitter dopamine from the synaptic cleft of dopaminergic neurons, back into the presynaptic neuron, and thus terminates dopamine neurotransmission (Amara, & Kuhar, 1993; Ciliax, Drash, Staley, Haber, Mobley, Miller, et al., 1999; Giros, Mestikawy, Godinot, Zheng, Han, Yang-Feng, et al., 1992). Vandenbergh, Persico, Hawkins, Griffin, Li, Jabs, et al. (1992) reported on a variable number of tandem repeats (VNTR) polymorphism in the 3’ untranslated region (UTR) of the DAT1 gene (henceforth referred to as the DAT1 3’ uVNTR). This polymorphism consists of a 40 base pair sequence repeated a variable number of times. The most common alleles consist of 10 repeats and 9 repeats respectively (Agudelo, Gálvez, Fonseca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Doucette-Stamm, Blakely, Tian, Mockus, & Mao, 1995; Gizer, Ficks, & Waldman, 2009; Kang, Palmatier, & Kidd, 1999; Santovito, Cervella, Selvaggi, Caviglia, Burgarello, Sella, et al., 2008). Since this polymorphism is not in the coding region of the DAT1 gene, it cannot have any effect on the protein sequence of the DAT transporter. It may, however, affect dopamine levels indirectly by altering translational efficiency and the subsequent amount of protein expressed (Bidwell, Willcutt, McQueen, DeFries, Olson, Smith, et al., 2011).

Many studies have been performed to determine whether the DAT1 3’ uVNTR does indeed affect the transcription and/or translation of the DAT1 gene (see Willeit, & Praschak-Rieder, 2010 for a review). Results have been mixed, with a clear distinction especially between results from in-vitro and in-vivo studies. The majority of in-vitro studies performed
on the effect of the DAT1 3’ uVNTR on gene expression, found the 10-repeat allele to be associated with greater expression of the DAT protein (Brookes, Neale, Sugden, Khan, Asherson, & D’Souza, 2007; Fuke, Suo, Takahashi, Koike, Sasagawa, & Ishiura, 2001; Mill, Asherson, Browes, D’Souza, & Craig, 2002; VanNess, Owens, & Kilts, 2005; Willeit, & Praschak-Rieder, 2010). Although far in the minority, a few in-vitro studies did find the 9-repeat allele to be associated with greater DAT expression (Miller, & Madras, 2002). In addition, a few in-vitro studies have also reported no association between the DAT1 3’uVNTR and DAT1 gene expression (Mill, Asherson, Craig, & D’Souza, 2005; Pinsonneault, Han, Burdick, Katak, Bertolino, Malhotra, et al., 2011).

In contrast to in-vitro study findings, findings from in-vivo studies on the effect of the DAT1 3’ uVNTR on gene expression have shown either the 9-repeat allele to be associated with increased availability/binding of the DAT protein (Faraone, Spencer, Madras, Zhang-James, & Biederman, 2014; Jacobsen, Staley, Zoghbi, Seibyl, Kosten, Innis, et al., 2000; Van de Giessen, De Win, Tanck, Van den Brink, Baas, & Booij, 2009; Van Dyck, Malison, Jacobsen, Seibyl, Staley, Laruelle, et al., 2005), or failed to find any association between this polymorphism and DAT availability/binding (J. Krause, Dresel, K.-H. Krause, Fougère, Zill, & Ackenheil, 2006; Lafuente, Bernardo, Mas, Crescenti, Aparici, Gassó, et al., 2007; Lynch, Mozley, Sokol, Maas, Balcer, & Siderowf, 2003; Martinez, Gelernter, Abi-Dargham, Van Dyck, Kegeles, Innis, et al., 2001). Given these conflicting findings, it is not surprising that Willeit, and Praschak-Rieder (2010) concluded in their review that the functional nature of the DAT1 3’ uVNTR is still not clear.

That said, overall, the evidence does seem to point to the DAT1 3’ uVNTR playing a role in the expression of the DAT1 gene. In turn, there is evidence that the expression of the DAT1 gene, and the consequent availability of the DAT protein, play an important role in controlling synaptic dopamine levels (Faraone, Spencer, Madras, Zhang-James, & Biederman, 2014; Fuke, Suo, Takahashi, Koike, Sasagawa, & Ishiura, 2001; Jaber, Jones, Giros, & Caron, 1997). Homozygous DAT knockout mice showed significant adaptive changes such as a decreased content of dopamine in presynaptic terminals, as well as decreased receptor levels. Dopamine also persisted at least 100 times longer outside the cells in these mice (Fuke, Suo, Takahashi, Koike, Sasagawa, & Ishiura, 2001; Jaber, Jones, Giros, & Caron, 1997). From these findings, Fuke, Suo, Takahashi, Koike, Sasagawa, and Ishiura (2001) concluded
that DAT does play an important role in the neurotransmission of dopamine, and that changes in the expression of DAT by the DAT1 gene profoundly influences dopaminergic pathways. This makes the DAT1 gene a plausible candidate gene in the aetiology of ADHD.

In addition, as already noted under section 2.5.1, MPH, the first line of pharmacological treatment for ADHD, exerts its therapeutic effect by blocking DAT in the human brain, resulting in an increase of extracellular dopamine (see section 2.5.1). This presents crucial evidence that the DAT1 gene, and consequently its gene product, the DAT protein, play a role in the aetiology of ADHD.

Considering the above, it is not surprising that numerous studies have examined the possible influence of the DAT1 gene, and in particular, the DAT1 3′ uVNTR polymorphism on ADHD. The first study reporting on an association between the DAT1 3′ uVNTR and ADHD was conducted by Cook, Stein, Krasowski, Cox, Olkon, Kieffer, et al. (1995). These researchers found a significant association between the 10-repeat allele of the DAT1 3′ uVNTR polymorphism and ADHD in 56 nuclear families. In subsequent studies, results have been conflicting. In a meta-analysis by Li, Sham, Owen, and He (2006), no significant association could be found between the 10-repeat allele of the DAT1 3′ uVNTR and ADHD. In a subsequent comprehensive meta-analysis by Gizer, Ficks, and Waldman (2009), the results of 35 studies conducted between 1995 and 2009, including the Cook, Stein, Krasowski, Cox, Olkon, Kieffer, et al. (1995) study, were pooled and odds ratios calculated. The 10-repeat allele of the DAT1 3′ uVNTR was considered as the risk allele. Bidwell, Willcutt, McQueen, DeFries, Olson, Smith, et al. (2011) described the odds ratios as representing “the magnitude of the association between ADHD and the putative risk alleles”. An odds ratio of 1.0 is indicative of no association, whilst an odds ratio bigger than 1.0 indicates that the allele increased the risk of developing ADHD. Gizer, Ficks, and Waldman (2009) reported an odds ratio of 1.12 for the 10-repeat allele, indicating a modest but significant association between this allele and ADHD. Apart from this positive association, two reviews, one conducted by Faraone, and Mick (2010) and the other by Gatt, Burton, Williams, and Schofield (2015), reported on considerable heterogeneity of findings across studies of the DAT1 3′ uVNTR polymorphism’s effect on ADHD. This high degree of heterogeneity of findings led both Faraone, and Mick (2010) and Gizer, Ficks, and Waldman (2009) to allude to the possibility
of a moderating environmental factor interacting with this polymorphism to influence ADHD. This will be covered later in the review (see section 2.7.6).

2.5.2.2 The Dopamine D4 receptor gene (DRD4)

The DRD4 gene has been mapped to the short arm of chromosome 11 (11p15.5), and is located close to the Harvey Ras oncogene (HRAS) (Gelernter, Kennedy, van Tol, Civelli, & Kidd, 1992). Several lines of evidence suggested the possible involvement of DRD4 in ADHD. DRD4 receptors are expressed in high concentrations in the prefrontal cortex, as indicated by studies on both mice and humans (Khan, Gutiérrez, Martín, Peñafiel, Rivera, & De La Calle, 1998; Lauzon, & Laviolette, 2010; Noaín, Avale, Wedemeyer, Calvo, Peper, & Rubinstein, 2006). In 1998, Faraone and Biedermann implicated the frontal cortex and areas projecting to the frontal cortex in the pathophysiology of ADHD (Faraone, & Biederman, 1998). More than 20 years later, this early suspicion was confirmed with a meta-analysis by Hart, Radua, Nakao, Mataix-Cols, and Rubia (2013) showing consistent functional abnormalities in the frontal cortex in patients diagnosed with ADHD.

Another possible link between DRD4 and ADHD is based on the reported connection between DRD4 and the personality trait of novelty seeking (Faraone, & Biederman, 1998; Gizer, Ficks, & Waldman, 2009; Gören, 2017). People who are high in novelty seeking display many of the same behaviours as those seen in ADHD (Faraone, & Biederman, 1998; Gizer, Ficks, & Waldman, 2009). The first positive association between DRD4 and novelty seeking was reported by Ebstein, Novick, Umansky, Priel, Osher, Blaine, et al. (1996), but results of a positive association in subsequent studies were inconsistent (Becker, Laucht, El-Faddagh, & Schmidt, 2005; Ekelund, Lichtermann, Järvelin, & Peltonen, 1999; Rogers, Joyce, Mulder, Sellman, Miller, Allington, et al., 2004; Schinka, Letsch, & Crawford, 2002; Sullivan, Fifield, Kennedy, Mulder, Sellman, & Joyce, 1998). However, a meta-analysis by Munafò, Yalcin, Willis-Owen, and Flint (2008) confirmed the probability of a link between the gene and novelty seeking.

The most frequently studied polymorphism in relation to ADHD in the DRD4 gene is a 48 base pair variable number of tandem repeats (VNTR) polymorphism in exon III of the gene (henceforth referred to as the DRD4 exon III VNTR) (Faraone, & Mick, 2010; Leung, Chan, Chen, Lee, Hung, Ho, et al., 2017; Lichter, Barr, Kennedy, Van Tol, Kidd, & Livak, 1993;
Niko laidis, & Gray, 2010; Pappa, Mileva-Seitz, Szekely, Verhulst, Bakermans-Kranenburg, Jaddoe, et al., 2014; Stanley, Chavda, Subramanian, Prabhu, & Ashavaid, 2017; Stergiakouli, & Thapar, 2010; Wu, Xiao, Sun, Zou, & Zhu, 2012). The DRD4 exon III VNTR is highly polymorphic, with repeats of the 48 base pair sequence varying from 2 to 11, commonly referred to as D4.2 to D4.11 (Wu, Xiao, Sun, Zou, & Zhu, 2012). The D4.4, D4.7 and D4.2 alleles of the DRD4 exon III VNTR have been found to be the most frequently occurring alleles worldwide (Chang, J.R. Kidd, Livak, Pakstis, & K.K. Kidd, 1996; K.K. Kidd, Pakstis, & Yun, 2014). However, allele frequencies differ significantly across populations (Chang, J.R. Kidd, Livak, Pakstis, & K.K. Kidd, 1996; Wang, Ding, Flodman, J.R. Kidd, K.K. Kidd, Grady, et al., 2004). Due to the discrepancy in allele frequencies across populations, caution should be exercised when attempting to perform association studies between a disorder and this polymorphism Chang, J.R. Kidd, Livak, Pakstis, & K.K. Kidd, 1996).

Evidence regarding the functionality of the DRD4 exon III VNTR is contradictory. Some studies have shown that the various repeat sequences affect the expression of the gene differently (Schoots, & Van Tol, 2003), whereas other studies have found no statistically significant relationship between a particular genotype and gene expression (Simpson, Vetuz, Wilson, Brookes, & Kent, 2010). Despite this uncertainty regarding the exact mechanism of action, several meta-analyses have indicated a link between the DRD4 exon III VNTR and ADHD (Faraone, Perlis, Doyle, Smoller, Goralnick, Holmgren, et al., 2005; Faraone, Doyle, Mick, & Biederman, 2001; Faraone, & Mick, 2010; Gizer, Ficks, & Waldman, 2009; Li, Sham, Owen, & He, 2006). In particular, the presence of the 7-repeat allele of the VNTR (D4.7) has been linked to an increased risk of having ADHD in several association studies and meta-analyses (Faraone, Biederman, Weiffenbach, Keith, Chu, Weaver, et al., 1999; Faraone, Perlis, Doyle, Smoller, Goralnick, Holmgren, et al., 2005; Faraone, Doyle, Mick, & Biederman, 2001; Faraone, & Mick, 2010; Gizer, Ficks, & Waldman, 2009; Gornick, Addington, Shaw, Bobb, Sharp, Greenstein, et al., 2007; Li, Sham, Owen, & He, 2006). In addition, studies have found links between the 4-repeat allele (D4.4) and ADHD (Shahin, Meguid, Raafat, Dawood, Doss, Bader el Din, et al., 2015; Tabatabaei, Amiri, Faghfouri, Noorazar, AbdollahiFakhim, & Fakhari, 2017), as well as between the 2-repeat allele (D4.2) and ADHD (Leung, Chan, Chen, Lee, Hung, Ho, et al., 2017). However, not all studies showed a positive association. A number of nationality-specific studies failed to find an association between the DRD4 exon
Ill VNTR and ADHD. For example, the VNTR was not associated with ADHD in samples from the Irish population (Hawi, McCarron, Kirley, Daly, Fitzgerald, & Gill, 2000), the Dutch population (Bakker, Van der Meulen, Oteman, Schelleman, Pearson, Buitelaar, et al., 2005), the Taiwanese population (Brookes, Xu, Chen, Huang, Wu, & Asherson, 2005), the Indian population (Stanley, Chavda, Subramanian, Prabhu, & Ashavaid, 2017), or the Norwegian population (Johanssone, Halleland, Halmøy, Jacobsen, Landaas, Dramsdahl, et al., 2008).

2.5.2.3 The Dopamine Beta-hydroxylase gene (DβH)

S.P. Craig, Buckle, Lamouroux, Mallet, and I.W. Craig (1988) mapped the DβH gene to chromosome 9q34. The DβH gene encodes the enzyme dopamine-β-hydroxylase (DβH), which is primarily responsible for catalysing the synthesis of norepinephrine from dopamine (Cubells, & Zabetian, 2004; Kaufman, & Friedman, 1965). A meta-analysis conducted by Scassellati, Bonvicini, Faraone, and Gennarelli (2012) on biomarkers in ADHD found decreased activity levels for DβH in serum and urine of ADHD patients, providing a rationale for the involvement of DβH, and consequently the DβH gene, in ADHD.

Although various polymorphisms in the DβH gene have been studied for possible association with ADHD (Roman, Schmitz, Polanczyk, Eizirik, Rohde, & Hutz, 2002; Smith, Daly, Fischer, Yiannoutsos, Bauer, Barkley, et al., 2003; Tong, McKinley, Cummins, Johnson, Matthews, Vance, et al., 2015; Zhang, Y.F. Wang, Li, B. Wang, & Yang, 2005), meta-analyses only found a significant association between a single nucleotide polymorphism (SNP) in intron 5 of the DβH gene and ADHD in children (Faraone, Perlis, Doyle, Smoller, Goralnick, Holmgren, et al., 2005; Gizer, Ficks, & Waldman, 2009). This SNP results in a Taq1 restriction site (rs2519152) and has not been found to be associated with levels of plasma DβH (Zabetian, Buxbaum, Elston, Köhnke, Anderson, Gelernter, et al., 2003). The first study to report an association between the Taq1 SNP and ADHD was conducted by Daly, Hawi, Fitzgerald, and Gill (1999). These researchers found that, after amplification of the SNP containing region of the DβH gene, digestion by the Taq1 restriction enzyme resulted in two alleles. The first allele, designated as “A1”, consisted of an undigested band of 464 base pairs, whilst the second allele, designated as “A2”, consisted of two bands of 300 base pairs and 164 base pairs respectively. In this study, the A2 allele was preferentially transmitted from parents to their affected offspring (Daly, Hawi, Fitzgerald, & Gill, 1999). This finding
was replicated by Roman, Schmitz, Polanczyk, Eizirik, Rohde, and Hutz (2002). These researchers also found over-transmission of the A2 allele in a sample of 88 nuclear families. In contrast, a study by Smith et al. (2003) found that the A1 allele of this polymorphism confers risk for ADHD. Further complicating these findings, studies by Wigg, Zai, Schachar, Tannock, Roberts, Malone, et al. (2002), Inkster, Muglia, Jain, and Kennedy (2004), Bhaduri, and Mukhopadhyay (2006), and Tong, McKinley, Cummins, Johnson, Matthews, Vance, et al. (2015) all found no significant association between any of the alleles of the Taq1 SNP and ADHD.

2.5.2.4 The Catachol-O-methyltransferase gene (COMT)

Catachol-O-methyltransferase (COMT) is one of the key enzymes responsible for metabolising monoamine neurotransmitters, including dopamine, in the prefrontal cortex of the human brain (Chen, Lipska, Halim, Ma, Matsumoto, Melhem, et al., 2004; Qian, Liu, Wang, Yang, Guan, & Faraone, 2009). The gene encoding the COMT enzyme (the COMT gene) has been mapped to chromosome 22q11 (Winqvist, Lundström, Salminen, Laatikainen, & Ulmanen, 1992). In line with the dopamine hypothesis of ADHD, the key role of the COMT enzyme in the degradation of dopamine makes the COMT gene a particularly attractive candidate gene for studies of this disorder. A common SNP at codon 158 of the COMT gene has been shown to affect enzymatic abundance and activity. This functional polymorphism (henceforth referred to as Val158Met) (rs4680) consists of a G to A transition, which results in the replacement of the amino-acid Valine with Methionine. The Met allele has been shown to be the low activity allele, resulting in a three to four-fold decrease in enzymatic activity, and subsequently in slower inactivation of dopamine in the brain. The Val allele, in contrast, results in higher enzymatic activity, especially in the prefrontal cortex (Chen, Lipska, Halim, Ma, Matsumoto, Melhem, et al., 2004; Lachman, Papolos, Saito, Yu, Szumlanski, & Weinshilboum, 1996; Levy, 2007; Qian, Liu, Wang, Yang, Guan, & Faraone, 2009).

Theoretically then, as summarised in a meta-analysis by Cheuk, and Wong (2006), the higher activity Val allele may result in increased degradation of dopamine in the synapses and a resultant lower concentration of dopamine compared to the Met allele. It is thus hypothesised that the Val allele would be associated with ADHD (Cheuk, & Wong, 2006). In the meta-analysis conducted by Cheuk, and Wong (2006), the data from 12 studies examining...
the effect of the Val158Met polymorphism on ADHD were pooled together. In this meta-analysis, no significant association was found between the Val158Met polymorphism of the COMT gene and ADHD. Similarly, a later meta-analysis conducted by Gizer, Ficks, and Waldman (2009), in which the relationship between ADHD and the Val158Met polymorphism was examined across 16 studies, also found no significant association. In two recent meta-analyses (Lee, & Song, 2015; Sun, Yuan, Shen, Xiong, & Wu, 2013) on 16 and 18 studies respectively, confirmed these findings, with no association detected between the Val158Met polymorphism and ADHD. In the meta-analysis by Sun, Yuan, Shen, Xiong, and Wu (2013), the researchers cautioned that the negative findings should not be viewed as definitive, since sample heterogeneity may have played an important role. In line with this, Shimada, Fujisawa, Takiguchi, Naruse, Kosaka, Okazawa, et al. (2017) found ethnic differences in the COMT Val158Met polymorphism’s influence on striatal abnormalities in Caucasian and Asian children with ADHD.

In addition, Cheuk, and Wong (2006) suggested that variation in the Val158Met polymorphism might be associated with ADHD in males only. Subsequent studies have supported this finding. Both Biederman, Kim, Doyle, Mick, Fagerness, Smoller, et al. (2008) and DeYoung, Getchell, Koposov, Yrigollen, Haeffel, Af Klinteberg, et al. (2010) found significant associations between the Met allele of the Val158Met polymorphism and ADHD in males. Qian, Liu, Wang, Yang, Guan, and Faraone (2009) found a significant association between ADHD with comorbid oppositional defiant disorder (ODD) and homozygosity of the Val allele in males. However, in the same study, Qian, Liu, Wang, Yang, Guan, and Faraone (2009) also found that the low activity Met allele was associated with the predominately inattentive ADHD subtype. In contrast, in a study on 4 101 individuals from the 1993 Pelotas Birth Cohort Study, Akutagava-Martins, Salatino-Oliveira, Kieling, Genro, Polanczyk, Anselmi, et al. (2016) found the Val allele to be associated with both hyperactivity and inattention scores in boys only. Thus, although the evidence pointed to the COMT Val158Met polymorphism playing a role in ADHD in males, more studies are needed to exactly pinpoint this association.

Interestingly, a number of studies have found that the Val/Val genotype of the Val158Met polymorphism was associated with increased symptoms of conduct disorder in people diagnosed with ADHD (Caspi, Langley, Milne, Moffitt, O'Donovan, Owen, et al., 2008;
Monuteaux, Biederman, Doyle, Mick, & Faraone, 2009; Salatino-Oliveira, Genro, Guimarães, Chazan, Zeni, Schmitz, et al., 2012; Thapar, Langley, Fowler, Rice, Turic, Whittingter, et al., 2005). It is therefore apparent that, as stated by Stergiakouli, and Thapar (2010), genetic variants such as the Val158Met polymorphism of the COMT gene can modify the phenotype of ADHD without actually increasing the risk of having the disorder itself.

2.5.2.5 The Monoamine oxidase A gene

The Monoamine oxidase A gene (MAOA) has been mapped to the short arm of the X chromosome (Xp11.3) (Levy, Powell, Buckle, Hsu, Breakefield, & Craig, 1989). The MAOA gene codes for the monoamine oxidase A enzyme (MAOA). MAOA has been found to catalyse the degradation of biogenic amines, including dopamine (Bortolato, Chen, & Shih, 2008; Chen, Hotamisligil, Huang, Wen, Ezzeddine, Aydin-Muderrisoglu, et al., 1991). Evidence associating MAOA with ADHD mainly came from a single study conducted by Brunner, Nelen, Breakefield, Ropers, and Van Oost (1993). In this study, the researchers found a point mutation in exon eight of the MAOA gene in a large Dutch family. This point mutation resulted in complete and selective deficiency in MAOA enzymatic activity in affected males in this family. The affected males displayed marked impulsive behaviours, including aggressive outbursts, arson, attempted rape, and exhibitionism. In addition, medication that inhibits MAOA activity has shown some success in the treatment of children with ADHD (Bonnet, 2003; Zametkin, Rapoport, Murphy, Linnoila, & Ismond, 1985), implicating a possible role for MAOA in the disorder.

Results from studies examining the possible role of various polymorphisms in the MAOA gene on ADHD have been inconsistent [for reviews see Banaschewski, Becker, Scherag, Franke, and Coghill (2010) and Faraone, and Mick (2010)]. In particular, attention has been paid to a variable number of tandem repeats (VNTR) polymorphism in the promoter region of the MAOA gene, first described by Sabol, Hu, and Hamer (1998). Now commonly referred to as the MAOA-uVNTR, this polymorphism is located 1.2 kb upstream of the MAOA coding sequences and consists of a 30 base pair sequence that is repeated a variable number of times (Sabol, Hu, & Hamer, 1998). Studies to date have shown this VNTR to be present in 2, 3, 3.5, 4 and 5 copies of the 30 base pair repeat sequence (Choi-Kwon, Ko, Jun, Kim, Cho, Nah, et al., 2017; Gizer, Ficks, & Waldman, 2009; Laubscher, Odendaal, Schneider, & Spies,
2012). The 3- and 4-repeat alleles are the most frequently observed (Ficks, & Waldman, 2014; C.F. Hung, Lung, T.H. Hung, Chong, Wu, Wen, et al., 2012). Sabol, Hu, and Hamer (1998) found more efficient transcription of alleles that contain 3.5 to 4 copies of the VNTR, compared to alleles containing 3 or 5 copies. Deckert, Catalano, Syagailo, Bosi, Okladnova, Di Bella, et al. (1999) agreed with the functional nature of the polymorphism, but found that alleles containing 5 copies, along with alleles containing 3.5 and 4 copies, were transcribed more effectively than the shorter 3 copy allele.

The first study to examine the association between the MAOA-uVNTR and ADHD was conducted by Manor, Tyano, Mel, Eisenberg, Bachner-Melman, Kotler, et al. (2002). In this study, the researchers found that the longer alleles of this polymorphism (3.5, 4 and 5 repeats) conveyed risk for ADHD. Making use of this initial finding, Gizer, Ficks, and Waldman (2009), in a meta-analysis, designated these longer alleles as the risk alleles. Pooling data from six studies, these researchers found no significant association between ADHD and these risk alleles. Following the results of this meta-analysis, few subsequent studies looking at a main effect of the MAOA-uVNTR on ADHD have been conducted. One study conducted by El-Tarras and colleagues in 2012 showed a positive association between the 3/4 and 3/2 MAOA-uVNTR genotypes and ADHD in a sample from Saudi Arabia (El-Tarras, Alsulaimani, Awad, Mitwaly, Said, & Sabry, 2012). It is thus clear that studies on the effect of this polymorphism on ADHD have to date yielded conflicting findings.

2.5.2.6 The Dopamine D2 receptor gene (DRD2)

The human dopamine D2 receptor gene (DRD2) has been mapped to the long arm of chromosome 11 (11q.23) (Eubanks, Djabali, Selleri, Grandy, Civelli, McElligott, et al., 1992; Grandy, Marchionni, Makam, Stofko, Alfano, Frothingham, et al., 1989). DRD2 codes for the human dopamine D2 receptor (Grandy, Marchionni, Makam, Stofko, Alfano, Frothingham, et al., 1989). The DRD2 receptor is a G-protein coupled receptor and has been found to inhibit adenylyl cyclase (Gizer, Ficks, & Waldman, 2009; Tang, Todd, Heller, & O’Malley, 1994).

When comparing results with that of the published sequence, latter researchers found that one cytosine was deleted in a run of two cytosines. This polymorphism will henceforth be referred to as the -141C Ins/Del polymorphism. Jönsson, Nöthen, Grünhage, Farde, Nakashima, Propping, et al. (1999) made use of positron emission tomography and found that individuals carrying the -141C Del allele had significantly higher striatal DRD2 receptor density than individuals carrying the -141C Ins allele. Providing further strength to the possibility that this polymorphism is functional, Arinami, Gao, Hamaguchi, and Toru (1997) showed that the -141C Del allele and the -141C Ins allele differed in terms of in vitro luciferase activity in Y-79 and 293 cells. Taken together, these findings point to a possible role for the DRD2 gene, and in particular the -141C Ins/Del polymorphism, in affecting symptoms of ADHD.

Apart from the -141C Ins/Del polymorphism, another possibly functional single nucleotide polymorphism (SNP) has also been identified in the 3’ untranslated region of the DRD2 gene (rs1800497). Commonly referred to as DRD2 Taq1A, this SNP comprises two alleles, a T allele and a C allele (Grandy, Marchionni, Makam, Stofko, Alfano, Frothingham, et al., 1989; Hemmings, Martin, Klopper, Van der Merwe, Aitken, De Wit, et al., 2013). In accordance with previous publications, these alleles will henceforth be referred to as the A1 and A2 alleles respectively. Similar to the effects observed for the -141C Ins allele, studies have shown that the A1 allele of the DRD2 Taq1A is associated with a reduction of DRD2 receptors in the brain (Hemmings, Martin, Klopper, Van der Merwe, Aitken, De Wit, et al., 2013; Noble, Blum, Ritchie, Montgomery, & Sheridan, 1991). As previously mentioned, reduced DRD2 receptor density has been linked to impulsivity, a prominent symptom of ADHD.

However, more direct evidence linking the DRD2 Taq1A to ADHD is also available. For example, a study by Serý, Drtílková, Theiner, Pitelová, Staif, Znojil, et al. (2006) found an association between the A1 allele, as well as the A1/A1 genotype, and ADHD in male subjects. That said, studies have also shown no preferential transmission of the DRD2 Taq1A alleles to children diagnosed with ADHD (Kirley, Hawi, Daly, McCarron, Mullins, Millar, et al., 2002). In a meta-analysis including six studies, Gizer, Ficks, and Waldman (2009) concluded that the heterogeneity in findings between the studies were of such a magnitude that no definite conclusions could be drawn regarding the role of the DRD2 Taq1A polymorphism in ADHD.
Adding to the heterogeneity in findings, a recent meta-analysis by Pan, Qiao, Xue, and Fu (2015) of 11 studies found that the A1 allele is significantly associated with risk for ADHD. Latter finding is in line with the findings of a meta-analysis conducted by Wu, Xiao, Sun, Zou, and Zhu (2012), in which a significant association was also found between the A1 allele and ADHD. However, similar to Gizer, Ficks, and Waldman (2009), Wu, Xiao, Sun, Zou, and Zhu (2012) noted excessive heterogeneity in the findings, and even went so far as to say that the positive association found needed to be disregarded and the causes of the heterogeneous findings sought.

2.5.2.7 The Dopamine D5 receptor gene (DRD5)

Similar to the dopamine D2 receptor, the dopamine D5 receptor is also a G-coupled receptor. However, in contrast to the D2 receptor, the D5 receptor stimulates adenylyl cyclase activity (Gizer, Ficks, & Waldman, 2009; Sunahara, Guan, O'Dowd, Seeman, Laurier, Ng, et al., 1991). The gene encoding the dopamine D5 receptor (DRD5) has been mapped to the short arm of chromosome four (4p15.1-p15.3) (Eubanks, Altherr, Wagner-McPherson, McPherson, Wasmuth, & Evans, 1992; Sherrington, Mankoo, Attwood, Kalsi, Curtis, Buetow, et al., 1993). A recent study on the spontaneously hypertensive rat (SHR) showed low dopamine D5 density in the hippocampus of these animals (Medin, Rinholm, Owe, Sagvolden, Gjedde, Storm-Mathisen, et al., 2013). Since the SHR is viewed as one of the best animal models for ADHD (Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005), this finding points to a possible connection between the dopamine D5 receptor and ADHD.

Sherrington, Mankoo, Attwood, Kalsi, Curtis, Buetow, et al. (1993) identified a highly polymorphic microsatellite marker consisting of a dinucleotide repeat sequence ((CA)n) 18.5kb 5’ to the DRD5 gene on chromosome four. They identified 12 alleles, ranging in size from 135 to 156 base pairs. The 148 base pair allele was identified as the most common. Daly, Hawi, Fitzgerald, and Gill (1999) were the first researchers to identify an association between this polymorphism and ADHD. In a sample of 118 Irish children and 200 of their parents, the 148 base pair allele was preferentially transmitted to affected children. This finding was replicated in four subsequent independent meta-analyses (Gizer, Ficks, & Waldman, 2009; Li, Sham, Owen, & He, 2006; Maher, Marazita, Ferrell, & Vanyukov, 2002;
Wu, Xiao, Sun, Zou, & Zhu, 2012), as well as in a study by Lowe, Kirley, Hawi, Sham, Wickham, Kratochvil, et al. (2004) consisting of a large combined homogeneous international sample.

It should be noted, however, that not all studies found a significant association between the 148 base pair allele of the DRD5 microsatellite and ADHD. For example, in a sample of Korean children, Kim, Kang, Cho, Park, Lim, Chung, et al. (2009) showed that the 142 and 144 base pair alleles, rather than the 148 base pair allele, were preferentially transmitted to children with ADHD. In addition, studies by Mill, Curran, Richards, Taylor, and Asherson (2004) and Fonseca, Mateus, Gálvez, Forero, Talero-Gutierrez, and Velez-van-Meerbeke (2015) found no association between any of the alleles of this microsatellite and ADHD.

2.6 A role for family studies in ADHD

Family studies are frequently the first line of enquiry when looking at possible genetic influences on a trait or disorder. However, there are a few limitations to family studies. There will be similarities between first-degree relatives for that trait either when additive genetic effects are present, or if the trait is influenced by factors in the shared environment. Non-additive genetic effects will not necessarily make first-degree relatives more similar to each other. Thus, lack of similarity between relatives for a particular trait could be due to the absence of genetic or shared environmental influence on the trait, but could also be the result of only non-additive genetic factors influencing the trait (Nikolas, & Burt, 2010; Plomin, & Daniels, 2011). In addition, one of the main problems with family studies is that the influence of genes and the influence of the shared environment cannot be separated. Thus, similarities between family members cannot be interpreted as being solely due to the influence of either genetic factors or the shared environment (Plomin, & Daniels, 2011). This problem may not be as pertinent in the study of ADHD, as the evidence from twin studies, discussed below, have shown.

In contrast to family studies, twin studies, where monozygotic twins are compared to dizygotic twins, provide a means to elucidate the effects of the environment and genes on behaviour separately. This is because dizygotic twins share on average 50% of their segregating genes, whilst monozygotic twins share 100% of their segregating genes. Contrasting similarities in behaviour between monozygotic and dizygotic twin pairs can give
an indication of the relative environmental and genetic influences on the behaviour (Coolidge, Thede, & Young, 2000; Nikolas, & Burt, 2010; Willcutt, Pennington, & DeFries, 2000). This is because if additive genetic effects act alone to influence a trait, the correlations between monozygotic twins would approximately be twice as large as that between dizygotic twins. If dominant genetic effects act alone to influence a trait, the correlations between monozygotic twins would be more than twice as large as the correlations between dizygotic twins. In contrast, if shared environmental effects were acting alone on a trait, monozygotic and dizygotic twin correlations would be similar in magnitude. This is due to different degrees of genetic relatedness having no influence on shared environmental factors. Finally, nonshared environmental factors will result in a reduction in both monozygotic and dizygotic twin correlations to the same degree (Burt, 2009; Nikolas, & Burt, 2010). Importantly, a frequently replicated finding in the ADHD literature is that the influence of the shared environment seems to be negligible (Burt, 2009; Levy, Hay, & Bennett, 2006; Nikolas, & Burt, 2010). Thus, it can be assumed that any similarities between family members must be due to additive genetic variance. This makes family studies a viable form of research for determining the influence of genetic factors on ADHD.

Apart from helping to determine the magnitude of additive genetic influence in ADHD, family studies can also be useful in several other ways. As will be discussed below, family studies can be used in various ways to aid in finding reasons for the mixed findings found thus far in molecular genetic studies of ADHD.

2.7 Explaining the mixed findings in molecular genetic studies of ADHD

From the discussion on the molecular genetics of ADHD above, it should be clear that there is a high degree of conflicting results regarding the influence of candidate genes on ADHD. It is possible that various forms of ADHD, with different aetiological mechanisms, exist. Should this be the case, studies using samples heterogeneous for different forms of the disorder may yield conflicting results. For example, ADHD subtypes may represent distinct disorders rather than varying presentations of the same disorder; ADHD comorbid with other disorders may represent distinct disorders; and there may be familial and non-familial forms of ADHD (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015; Stawicki, Nigg, & Von
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Eye, 2006). Furthermore, mixed findings may also result from rare genetic variants sometimes influencing the disorder (Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012) from interactions between loci impacting ADHD (Brookes, Xu, Anney, Franke, Zhou, Chen, et al., 2008), and/or from environmental factors interacting with genes influencing the disorder (Ficks, & Waldman, 2009). Each of these possibilities will be explored further below.

2.7.1 Evidence for ADHD subtypes being distinct disorders or varying presentations of the same disorder

A point of great contention in the literature is whether ADHD inattentive type, ADHD combined type, and ADHD hyperactive-impulsive type are truly varying presentations of the same disorder or actually distinct disorders (Diamond, 2005; Faraone, Biederman, Mick, Williamson, Willens, Spencer, et al., 2000; Faraone, Biederman, & Friedman, 2000; Milich, Balentine, & Lynam, 2001; Nigg, Tannock, & Rohde, 2010; Smalley, McGough, Del’Homme, NewDelman, Gordon, Kim, et al., 2000; Stawicki, Nigg, & Von Eye, 2006; Todd, Rasmussen, Neuman, Reich, Hudziak, Bucholz, et al., 2001; Willcutt, Pennington, & DeFries, 2000; Woo, & Rey, 2005). Should subtypes be distinct disorders with different aetiological factors influencing each, studies using samples heterogenous for the subtypes may result in conflicting findings. Particular attention has been paid to the distinction between ADHD combined type and ADHD inattentive type, due to the low prevalence of ADHD hyperactive-impulsive type (Diamond, 2005; Faraone, Biederman, & Friedman, 2000; Lahey, 2001; Milich, Balentine, & Lynam, 2001; Stawicki, Nigg, & Von Eye, 2006). Milich, Balentine, and Lynam (2001) noted that up until the publication of the DSM-III in 1980, the occurrence of attention problems without hyperactivity-impulsivity was not mentioned in the literature. This explains the flurry of research to determine whether ADHD inattentive type is indeed a valid subtype of ADHD or a completely distinct disorder (Milich, Balentine, & Lynam, 2001).

Studies on the neurostructural, neurofunctional, neurocognitive, molecular genetic, and treatment response similarities/differences between ADHD combined type and ADHD inattentive type have been conducted to determine the combined or separate aetiological nature of these subtypes. In the neurological studies conducted, researchers have shown different microstructural white matter brain abnormalities (Lei, Ma, Du, Shen, Jin, & Gong,
different patterns of atypical neural connectivity (Fair, Nigg, Lyer, Bathula, Mills, Dosenbach, et al., 2013), different task-related neurophysiological impairments (Mazaheri, Fassbender, Coffey-Corina, Hartanto, Schweitzer, & Mangun, 2014), and different neurocognitive deficits (Dovis, Van der Oord, Wiers, & Prins, 2015) between the two subtypes. In all these studies, authors also found overlapping neural features between the two disorders, supporting the conclusion that the subtypes represent variations of the same disorder.

Although few studies have thus far been conducted on pharmacological treatment response differences between the subtypes, a recent study by Beery, Quay, and Pelham (2017) found that methylphenidate (MPH) was more beneficial in the reduction of symptoms for ADHD combined type compared to ADHD inattentive type, and in some cases even had a detrimental effect on problem behaviours in children with predominantly inattentive symptoms. This finding replicates a previous finding by Grizenko, Paci, and Joober (2010), who found a higher frequency of a beneficial MPH response in a group of children with either ADHD combined type or hyperactivity only, compared to an ADHD inattentive type group. However, in a comprehensive meta-analysis, Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al. (2012), concluded that there is little evidence for differential efficacy of treatment between the ADHD combined and ADHD inattentive subtypes.

In the same meta-analysis, Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al. (2012) reported that no studies included in the meta-analysis found a significant difference in the association of any candidate genes with ADHD combined type and ADHD inattentive type when the two subtypes were compared directly. Surprisingly, there are very few recent studies comparing molecular genetic factors between the ADHD subtypes, with the vast majority of molecular genetic studies not parsing samples into ADHD combined and ADHD inattentive subtypes (Gatt, Burton, Williams, & Schofield, 2015; Hawi, Cummins, Tong, Johnson, Lau, Samarrai, et al., 2015; Middeldorp, Hammerschlag, Ouwens, Groen-Blokhuys, St. Pourcain, Greven, et al., 2016). The one study that could be found compared genotype distributions between single nucleotide polymorphisms (SNPs) in the alpha-2A adrenergic receptor gene (ADRA2A) and the COMT gene between ADHD inattentive and ADHD combined subtypes, but no significant differences were found (D. Unal, M.F. Unal, Alikasifoglu, & Cetinkaya, 2016).
Apart from the above studies into the neural, genetic, and treatment response differences between ADHD combined and ADHD inattentive types, family studies can, and have been, used to explore whether these subtypes represent distinct disorders or varying presentations of the same disorder. As explained by Stawicki, Nigg, and Von Eye (2006), if the ADHD combined and ADHD inattentive subtypes are just variations of the same condition, relatives of children with ADHD combined type should be just as likely to have ADHD inattentive type as ADHD combined type, and vice versa. The subtypes should thus not “breed true” in families. This is often referred to as the “gradient-of-severity” hypothesis. If the converse is true, and the subtypes represent distinct disorders, relatives of children with ADHD combined type should be significantly more likely to have ADHD combined type than ADHD inattentive type, and vice versa. Thus, the subtypes would be expected to breed true in families (Faraone, Biederman, & Friedman, 2000; Stawicki, Nigg, & Von Eye, 2006).

Results from family studies testing the distinction between the subtypes have been conflicting, although the weight of the evidence points to ADHD combined type and ADHD inattentive type simply being variations of the same disorder. Studies classifying subtypes according to criteria stipulated in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (APA, 1994) found that the ADHD combined and inattentive subtypes do not breed true, with no association between the subtype of the affected child and that of his/her relatives (Faraone, Biederman, Mick, Williamson, Wilens, Spencer, et al., 2000; Faraone, Biederman, & Friedman, 2000; Smalley, McGough, Del'Homme, NewDelman, Gordon, Kim, et al., 2000). Two subsequent meta-analysis pooling data from multiple family studies for the most part confirmed these findings. In the first meta-analysis, Stawicki, Nigg, and Von Eye (2006) concluded that some degree of both subtype-specific transmission and non-specific (“gradient”) transmission do occur, although the effect size for non-specific transmission was small. Latter researchers proposed that ADHD combined type and ADHD inattentive type are partially overlapping conditions, but that they also have some distinct aetiological factors. In a subsequent meta-analysis, Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al. (2012) found that there was insufficient evidence to classify DSM-IV ADHD subgroups as distinct disorders, particularly citing the poor long-term stability that has been found for the subtypes.
Further research is thus needed to determine whether ADHD combined type and ADHD inattentive type are indeed just subtypes of the same disorder or aetiologically distinct disorders. As mentioned by Milich, Balentine, and Lynam (2001), if ADHD inattentive type is indeed a distinct disorder and not a true ADHD subtype, using samples in ADHD research that include participants with ADHD inattentive type may lead to flawed results. Therefore, this may be a possible explanation for the mixed findings. In addition, this type of study has never been conducted in a South African population.

2.7.2 The aetiological nature of ADHD comorbid with ODD

A possible explanation for the mixed findings may be due to ADHD being comorbid with other disorders, representing distinct forms of the disorder. Similar to ADHD, oppositional defiant disorder (ODD) has its onset in early childhood (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005). The DSM-5 (APA, 2013) defines ODD as a “pattern of angry/irritable behaviours and vindictiveness lasting at least six months”. The DSM 5 grouped eight core symptoms of ODD into three categories, namely angry/irritable mood, argumentative/defiant behaviour, and vindictiveness. The category of angry/irritable mood included symptoms such as often loses temper, tends to be touchy or easily annoyed, and tends to be angry or disrespectful. The category of argumentative/defiant behaviour included symptoms such as frequently argues with authority figures or adults, defies or refuses to comply with rules or requests from authority figures, deliberatelyannoys others, and often blames others.

ODD is associated with social impairment and family dysfunction (Greene, Biederman, Zerwas, Monuteaux, Goring, & Faraone, 2002), precedes conduct disorder, and is associated with an increased risk for later anxiety disorders, mood disorders, and antisocial personality disorder (Biederman, Petty, Dolan, Hughes, Mick, Monuteaux, et al., 2008; APA, 2013; Lavigne, Cicchetti, Gibbons, Binns, Larsen, & Devito, et al., 2001; Rowe, Maughan, Pickles, Costello, & Angold, 2002). ODD has been found to be one of the disorders most frequently co-occurring with ADHD, with studies from around the world showing comorbidity rates from 28% up to as high as 60% (Biederman, 2005; Connor, Steeber, & McBurnett, 2010; Cuffe, Visser, Holbrook, Danielson, Geryk, Wolraich, et al., 2015; Inci, Ipci, Akyol Ardıç, & Ercan, 2016; Joelsson, Chudal, Gyllenberg, Kesti, Hinkka-Yli-Salomäki, Virtanen, et al., 2016; Nock, Kazdin, Hiripi, & Kessler, 2007; Waschbusch, 2002). There is reliable evidence that the
Comorbid occurrence of ADHD and ODD is associated with poorer outcomes than if a child is diagnosed with only one of the two disorders (Connor, & Doerfler, 2008; Connor, Steeber, & McBurnett, 2010; Dalsgaard, Mortensen, Frydenberg, & Thomsen, 2002; Waschbusch, 2002). Connor, and Doerfler (2008) found that ADHD comorbid with ODD resulted in poorer educational outcomes, greater ADHD symptom severity, and greater levels of aggression and delinquency than either disorder diagnosed without the other. The high rates of comorbidity of these two disorders, along with the exacerbated negative outcomes compared to children only diagnosed with one of the two, have stimulated numerous research studies aiming to understand this comorbidity better (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su et al., 2008; Harvey, Breaux, & Lugo-Candelas, 2016; Vierikko, Pulkkinen, Kaprio, & Rose, 2004; Zenglein, Schwenck, Westerwald, Schmidt, Beuth, Meyer, et al., 2016).

Different hypotheses have been put forward to explain the co-occurrence of ADHD and ODD (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008; Hamshere, Langley, Martin, Agha, Stergiakouli, Anney, et al., 2013; Harvey, Breaux, & Lugo-Candelas, 2016; Tuvblad, Zheng, Raine, & Baker, 2009). Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) proposed four possible mechanisms for the co-occurrence of ADHD and ODD and/or conduct disorder (CD), based on published research (Faraone, Biederman, Jetton, & Tsuang, 1997; Greene, Biederman, Zerwas, Monuteaux, Goring, & Faraone, 2002; Schachar, & Tannock, 1995). In their paper, they also predicted the expected outcomes for siblings of affected probands for each mechanism. The researchers used the broad term conduct problems (CP) to describe the occurrence of either CD or ODD.

Firstly, it is possible that ADHD and ODD are aetiologically independent from one another. Thus, the two disorders only co-occur by chance (Hypothesis 1). Secondly, ADHD and ODD occurring together might be a distinct disorder from either ADHD or ODD occurring alone (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008; Hurtig, Ebeling, Taanila, Miettunen, Smalley, McGough, et al., 2007) (Hypothesis 2). Thirdly, ADHD plus ODD might be an extreme and severe variant of ADHD, with a greater genetic loading (Hypothesis 3). Finally, it is also possible that the co-occurrence of ADHD and ODD is due to the two disorders sharing common environmental risk factors (Hypothesis 4) (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008).
2.7.2.1 Hypothesis 1: Evidence that ADHD and ODD co-occur only by chance

Should ADHD and ODD co-occur due to chance alone, the following frequencies can be projected: The recurrence risk for ADHD only, ADHD plus ODD, and ODD only in siblings of probands with ADHD plus ODD should be low and more or less equal. In contrast, siblings of ADHD only probands should have a markedly higher risk to have ADHD only (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). However, as previously noted, the mere frequency with which ADHD and ODD co-occur makes this mechanism of comorbidity highly unlikely (Biederman, 2005; Connor, Steeber, & McBurnett, 2010; Waschbusch, 2002). In addition, the multitude of family studies and twin studies conducted all show definite factors influencing the co-occurrence of the two disorders (e.g. Burt, Krueger, McGue, & Iacono, 2001; Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al., 2009). Hypothesis 1 can thus be refuted as highly improbable.

2.7.2.2 Hypothesis 2: Evidence that ADHD and ODD co-segregate within families

Should ADHD plus ODD be a distinct disorder from either of the disorders occurring alone, it would be expected that relatives of individuals diagnosed with ADHD and ODD should also have higher rates of both ADHD and ODD, and not just higher rates of one of the disorders. In other words, the two disorders should co-segregate within families (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). Co-segregation is known as the tendency for disorders to be inherited together (Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al., 2009). To determine whether two disorders co-segregate, relatives of probands who have both disorders can be viewed as an informative sample, if both disorders have been shown to be familial. If the co-occurring disorder in the relatives of probands with both disorders is significantly more common amongst relatives who also have the other disorder than amongst relatives who do not have the other disorder, co-segregation of the two disorders is signalled (Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Geller, Petty, Vivas, Johnson, Pauls, & Biederman, 2007; Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al., 2009). Co-segregation of disorders within families also points to a distinct familial subtype of a disorder, rather than independently transmitted disorders (Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al., 2009).
For example, Biederman, Petty, Hammerness, Woodworth, and Faraone (2013) assessed the comorbidity of ADHD with nicotine dependence (ND) in a group of relatives of probands who had both ADHD and nicotine dependence. They found no statistically significant difference in the rates of nicotine dependence between relatives with ADHD and relatives without ADHD. This finding suggests that ADHD and ND do not co-segregate in families. Similar studies have been conducted to determine co-segregation between numerous other disorders and ADHD, including obsessive compulsive disorder (Geller, Petty, Vivas, Johnson, Pauls, & Biederman, 2007), anxiety disorders (Braaten, Biederman, Monuteaux, Mick, Calhoun, Cattan, et al., 2003), and antisocial disorders (Faraone, Biederman, Jetton, & Tsuang, 1997).

One study, conducted by Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al. (2009), looked at the familial association between ADHD and ODD outside the context of CD. Latter researchers studied relatives of probands diagnosed with ADHD plus ODD and found that ODD occurred significantly more frequently in the relatives who, like the probands, were also diagnosed with ADHD, than in relatives without ADHD. As explained earlier, this signals co-segregation of the two disorders in this group of families, and possibly points to ADHD plus ODD being a distinct familial disorder.

As noted, Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) used the construct “conduct problems” (CP), described as “a broad category that allows for the occurrence of CD and ODD”. They used a large sample of cases with ADHD and their unaffected siblings. The hypothesis was tested that ADHD plus CP is a distinct disorder, independent from ADHD alone, that co-segregates in families. The researchers postulated that cases diagnosed only with ADHD will tend to have siblings with only ADHD symptomology if the hypothesis is correct. In addition, cases with ADHD plus CP will tend to have siblings with both ADHD and CP symptomology. The researchers used multinomial logistic regression. The status of the cases as ADHD only or ADHD plus CP served as the predictor variable, whereas the status of the siblings was used as the criterion variable. The findings were similar to that found by Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al. (2009). In cases with ADHD plus CP, a nearly five-fold increased risk of siblings also showing symptoms of ADHD plus CP occurred, compared to cases with only ADHD. As in the Petty,
Monuteaux, Mick, Hughes, Small, Faraone, et al. (2009) study, these findings point to the co-segregation of ADHD and CP, and shows ADHD plus CP to be a distinct familial disorder.

2.7.2.3 Hypothesis 3: Evidence that ADHD plus ODD is an extreme variant of ADHD

If ADHD plus ODD represents an extreme variant of ADHD, the two disorders would be expected to share a common genetic and environmental aetiology. In addition, siblings of cases diagnosed with ADHD plus ODD will be likely to have ADHD plus ODD, but will also be likely to have only ADHD (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008).

Supporting this mechanism, Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) found that cases diagnosed with ADHD and comorbid CP, had a nearly 3-fold increased likelihood to have siblings who were diagnosed with ADHD only. Furthermore, Nadder, Rutter, Silberg, Maes, and Eaves (2002) found that the co-occurrence of ADHD and ODD/CD can mostly be explained by a common set of genes. However, latter researchers cautioned that this finding can mean one or both of two things. Firstly, it can mean that the two phenotypes are different manifestations of the same underlying genetic vulnerability. This would support the view that ADHD plus ODD is just a different manifestation of the same disorder caused by the same underlying genetic factors. Secondly, it is also possible that ADHD actually results in symptoms of ODD and CD. If this is the case, albeit misleading, the same result of ADHD and ODD/CD being caused by a similar underlying genetic liability could be expected.

In addition to the study by Nadder, Rutter, Silberg, Maes, and Eaves (2002), a number of studies have found that ADHD and conduct problems share a common genetic and environmental aetiology (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Thapar, Harrington, & McGuffin, 2001; Tuvblad, Zheng, Raine, & Baker, 2009; Vierikko, Pulkkinen, Kaprio, & Rose, 2004), thus supporting the hypothesis/suggestion that ADHD plus ODD is just a more severe form of the same disorder. That said, several studies (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Tuvblad, Zheng, Raine, & Baker, 2009; Vierikko, Pulkkinen, Kaprio, & Rose, 2004) described some degree of unique genetic influence for both ADHD and conduct problems, whilst Tuvblad, Zheng, Raine, and Baker (2009) found the influence of shared environmental factors to be non-significant. This suggests that there are grounds for categorising these disorders as separate disorders which are somewhat independent in their underlying
biological structures. Consequently, Dick, Viken, Kaprio, Pulkkinen, and Rose (2005) postulated that some genes are general risk factors for all externalising disorders, including ADHD and ODD, whilst other genes increase the risk of symptoms specific to the individual disorders.

Latter findings somewhat dispute the idea of ADHD plus ODD simply being an extension of the ADHD phenotype. A molecular genetic study by Comings, Gade-Andavolu, Gonzalez, Wu, Muhleman, Blake, et al. (2000) supports the findings by Dick, Viken, Kaprio, Pulkkinen, and Rose (2005) and Tuvblad, Zheng, Raine, and Baker (2009). In a study examining the effects of 20 genes, Comings, Gade-Andavolu, Gonzalez, Wu, Muhleman, Blake, et al. (2000) showed that ODD shared genes with ADHD, whilst simultaneously being influenced by genes different from those influencing ADHD.

2.7.2.4 Hypothesis 4: Evidence that ADHD plus ODD co-occur due to common environmental risk factors

If ADHD and CP are separate entities and co-occur due to shared environmental factors, siblings of probands diagnosed with ADHD only would be expected to have siblings with ADHD only. ADHD plus CP probands will have some siblings with ADHD only, and many siblings with ADHD plus CP and CP only (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008).

Previous studies have found evidence in support of the influence of shared environmental risk factors on the comorbidity between ADHD and ODD (Burt, Krueger, McGue, & Iacono, 2003, 2001; Burt, McGue, Krueger, & Iacono, 2005; Kuja-Halkola, Lichtenstein, D’Onofrio, & Larsson, 2015; Martin, Levy, Pieka, & Hay, 2006). It should be noted that all of these studies found genetic factors to also play a role in the co-occurrence of the disorders, and Kuja-Halkola, Lichtenstein, D’Onofrio, and Larsson (2015) found that stable shared environmental factors contributed to the comorbidity, but only in early adolescence. Relating to the nature of the shared environmental factors influencing this comorbidity, Burt, Krueger, McGue, and Iacono (2003) found that parent-child conflict serves as a common risk factor, increasing the vulnerability for child externalising disorders. The authors posit that underlying pathological processes in the family environment drives the co-occurrence of these otherwise distinct psychological disorders. In contrast to these findings,
numerous studies have found that the co-morbidity between ADHD and ODD are unlikely to be due to shared environmental influences (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Nadder, Rutter, Silberg, Maes, & Eaves, 2002; Tuvblad, Zheng, Raine, & Baker, 2009).

From the above discussion, it can be seen that apart from hypothesis 1, the literature provides ample evidence for the validity of all three of the other hypotheses. Since these hypotheses contradict one another, more research is clearly needed to elucidate the true cause of the frequent co-occurrence of ADHD and ODD.

2.7.3 The aetiological nature of simplex and multiplex ADHD

A further reason for the mixed findings may be that ADHD can be parsed into familial and non-familial forms. Samples heterogenous for these alternative forms of the disorder may then yield conflicting results. In this line of research, families with only one affected family member are referred to as simplex families. Multiplex families are families where at least two family members are affected by a disorder (Oerlemans, Burmanja, Franke, Buitelaar, Hartman, & Rommelse, 2016; Sullivan, Daly, & O’Donovan, 2012).

This distinction between simplex and multiplex families has been explored extensively in research on autism spectrum disorders (ASD), but for ADHD, this avenue of research remains largely unexplored (Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al., 2014; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015; Sullivan, Daly, & O’Donovan, 2012). Results from studies on simplex and multiplex ASD families have shown that different aetiological factors influence the simplex and multiplex forms of the disorder. Simplex ASD was found to be influenced mostly by risk factors unique to the individual, whereas multiplex ASD was influenced mainly by factors shared between multiple family members. Thus, if a disorder shows to have both simplex and multiplex forms, this enables researchers to segregate heterogenous populations into samples with only simplex families and samples with only multiplex families. The simplex family samples then enables researchers to focus on individual specific genetic and/or environmental causes, whereas multiplex family samples allow the study of polygenic and/or shared environmental causes (Freitag, 2007; Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al., 2014; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015; Oerlemans, Burmanje,

The limited number of studies that have explored the simplex/multiplex family distinction in ADHD have yielded conflicting results. In a study by Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al. (2014), the researchers compared behavioural traits associated with ADHD in unaffected family members from simplex, multiplex, and control families. The hypothesis was that unaffected members from simplex and multiplex families would differ in the symptoms they show, should simplex ADHD be caused by shared environmental/polygenetic factors, and complex ADHD by individual specific genetic/environmental factors. Unaffected members in multiplex families were expected to show a greater degree of ADHD symptoms than unaffected members in simplex families. However, their results refuted this hypothesis. Unaffected members from simplex and multiplex families all showed equally elevated levels of ADHD symptoms when compared to unaffected members from control families.

In a subsequent study, Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, and Rommelse (2015) investigated cognitive vulnerability profiles as an endophenotype of ADHD, rather than looking at ADHD traits directly. In contrast to the Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al. (2014) study, results showed that cognitive vulnerability profiles differed between simplex, multiplex, and control families. In multiplex ADHD families, unaffected members’ cognitive vulnerability profiles fell in between that shown by affected members and members from control families. For simplex ADHD families, however, the cognitive vulnerability profiles of unaffected members could not readily be distinguished from that of controls. This result is in line with the hypothesis that a distinction can be made between simplex and multiplex ADHD, with simplex ADHD influenced by factors unique to the individual, resulting in no symptoms in other family members. In contrast, multiplex ADHD results in all family members showing symptoms, whether affected or not, and is thus influenced by factors shared between family members.

Rare genetic variants unique to the individual have indeed been shown to influence ADHD, with de novo rare copy number variants (CNV’s) identified in ADHD related cases (Ehli, Abdellaoui, Hu, Hottenga, Kattenberg, Van Beijsterveldt, et al., 2012; Lionel, Crosbie,
Barbosa, Goodale, Thiruvahindrapuram, Rickaby, et al., 2011). In addition, environmental risk factors specific to the individual have also been found to influence ADHD (Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al., 2012; Oerlemans, Burmanje, Franke, Buitelaar, Hartman, & Rommelse, 2016). These environmental risk factors will be discussed later in this review.

2.7.4 Rare genetic variants and allelic heterogeneity

The molecular genetic studies described above were all based on the common disease/common variant (CDCV) hypothesis. This hypothesis postulates that high frequency diseases in the human population can be attributed to genetic variations that also occur at a high frequency in the population (Collins, 1997; Gibson, 2012; Schork, Murray, Frazer, & Topol, 2009). Although the results outlined above clearly provide credence to this hypothesis, the mixed results also indicate that common genetic variants cannot always explain the occurrence of ADHD.

One possible explanation for the mixed results is genetic variability within the described repeat sequences (Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012). This explanation forms part of a contrasting hypothesis named the common disease/rare variant (CDRV) hypothesis. This hypothesis postulates that a multitude of rare, mildly deleterious genetic variations with relatively high penetrance are the major underlying factors in common human disease (Pritchard, 2001; Schork, Murray, Frazer, & Topol, 2009; Smith, 2002; Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012). In the context of ADHD, the CDRV hypothesis assumes that rare genetic variants are major factors in the aetiology of ADHD, and that allelic heterogeneity could also have an important role to play in the disorder (Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012). However, it is likely that both common and rare genetic variants have a role to play in ADHD, rather than just one of the two (Martin, O’Donovan, Thapar, Langley, & Williams, 2015; Stergiakouli, Hamshere, Holmans, Langley, Zaharieva, ceCODE Genetics Psychiatric GWAS Consortium: ADHD Subgroup, et al., 2012).

The joint contribution of common and rare genetic variants to the aetiology of ADHD was recently studied by Martin, O’Donovan, Thapar, Langley, and Williams (2015). The researchers assumed that ADHD can be explained by means of a polygenic liability threshold
model. Aggregate scores of common genetic variants previously implicated in the aetiology of ADHD were calculated and given the name “polygenic risk scores”. These polygenic risk scores were then compared between individuals diagnosed with ADHD with rare risk alleles, and diagnosed individuals without rare risk alleles. In line with the CDRV hypothesis that rare genetic variants have relatively high penetrance in ADHD, Martin, O’Donovan, Thapar, Langley, and Williams (2015) found that diagnosed children who carried rare risk variants had lower polygenic risk scores than diagnosed children without rare variants. It is therefore possible that in some individuals, an aggregate of common genetic variants causes ADHD, whilst in other individuals, rare genetic variants play the most important role. Samples heterogeneous for individuals carrying only common genetic risk variants and individuals carrying rare mutations could therefore result in mixed findings, since the aetiological nature of the disorder would differ in these two groups.

One form of rare genetic variant for which research to date is lacking is DNA sequence variations within commonly observed VNTR polymorphisms. Only a limited number of studies to date have focused on sequence variations within VNTR’s of genes related to ADHD (Grady, Chi, Ding, Smith, Wang, Schuck, et al., 2003; Hawi, Cummins, Tong, Johnson, Lau, Samarrai, et al., 2015; Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012; Tovo-Rodrigues, Rohde, Menezes, Polanczyk, Kieling, Genro, et al., 2013). In the few studies conducted so far, the focus has solely been on the 48 base pair VNTR in exon 3 of the DRD4 gene (Grady, Chi, Ding, Smith, Wang, Schuck, et al., 2003; Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012; Tovo-Rodrigues, Rohde, Menezes, Polanczyk, Kieling, Genro, et al., 2013). Concerning genetic variability, the DRD4 gene is one of the most variable human genes, with this variability mostly attributable to single nucleotide polymorphisms (cSNP’s) and length variations of the 48 base pair repeat sequence in exon 3 (Chang, J.R. Kidd, Livak, Pakstis, & K.K. Kidd, 1996; Ding, Chi, Grady, Morishima, J.R. Kidd, K.K. Kidd, et al., 2002; Grady, Chi, Ding, Smith, Wang, Schuck, et al., 2003; Kidd, Pakstis, & Yun, 2014; Lichter, Barr, Kennedy, Van Tol, Kidd, & Livak, 1993; Martínez-Levy, Benjet, Briones-Velasco, Pérez-Molina, Nani, & Cruz-Fuentes, 2013). Using a world-wide sample, Ding, Chi, Grady, Morishima, J.R. Kidd, K.K. Kidd, et al. (2002) found 35 different base pair motifs of the 48 base pair repeat region, which were combined into 56 different haplotypes. Each of the different 48 base pair motifs was given a numeric value (1 to 35), and the haplotypes were indicated by the
The most common number of repeats found in alleles in the sample were the two repeat (2R), the four repeat (4R), and the seven repeat (7R) variants, with the following common haplotypes for each: 2R (1-4); 4R (1-2-3-4); 7R (1-2-6-5-2-5-4). In addition to these common haplotypes, population specific rare haplotypes were also found, for example a 2R (30-4) haplotype in the Surui for South America; and a 5R (1-3-2-3-4) haplotype found only in the Han Chinese from Asia (Ding, Chi, Grady, Morishima, J.R. Kidd, K.K. Kidd, et al., 2002).

A study by Grady, Chi, Ding, Smith, Wang, Schuck, et al. (2003) was the first to study the number of repeats of the 48 base pair VNTR in relation to ADHD, as well as sequence variations within the 48 base pair repeat motif. These authors proposed that the association between the 7R allele of the DRD4 exon III VNTR might be due to the 7R allele having a higher mutation rate than the other alleles, and that it is actually these mutated forms of the 7R that is associated with ADHD. Their results showed a higher than normal prevalence of the 7R allele in the ADHD sample. Although most of these 7R alleles were of the conserved haplotype previously identified by Ding, Chi, Grady, Morishima, J.R. Kidd, K.K. Kidd, et al. (2002), Grady, Chi, Ding, Smith, Wang, Schuck, et al. (2003) did find that over 10% of the individuals diagnosed with ADHD carried rare haplotypes of all alleles of the DRD4 exon III VNTR. This finding is highly statistically significant, with a very low probability that the high frequency of rare alleles in the ADHD probands occurred by chance. Thus, this was the first study to show that rare allelic variants are enriched in a sample of ADHD probands. Also of note is the finding by Grady, Chi, Ding, Smith, Wang, Schuck, et al. (2003) that more than 90% of these rare DRD4 alleles identified in the ADHD probands resulted in an altered amino acid sequence in the DRD4 protein when compared to the common allele. These findings led to the hypothesis that any allele different from the ancestral 4R (1-2-3-4) allele might play a role in ADHD since it potentially alters the amino acid sequences of the proteins, their biochemical functions, and consequently phenotype (Grady, Chi, Ding, Smith, Wang, Schuck, et al., 2003; Lichter, Barr, Kennedy, Van Tol, Kidd, & Livak, 1993; Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012; Wang, Ding, Flodman, J.R. Kidd, K.K. Kidd, Grady, et al., 2004).
In line with this hypothesis, Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al. (2012) investigated whether ADHD individuals showed a higher degree of variability at the *DRD4* exon III VNTR locus than controls in a Brazilian sample of 786 ADHD individuals. This was the first study, after Grady, Chi, Ding, Smith, Wang, Schuck, et al. (2003), to look at the relation between sequence variations within the 48 base pair repetitive sequence of the *DRD4* exon III VNTR and ADHD. The researchers found six variable sites within the 4R allele, and 31 variable sites within the 7R allele. The previously identified most frequent haplotypes, namely the 4R (1-2-3-4) and the 7R (1-2-6-5-2-5-4), were also the most frequent in the Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al. (2012) sample. Importantly, this study corroborated the findings by Grady, Chi, Ding, Smith, Wang, Schuck, et al. (2003) by also indicating an excess of rare haplotype variants in ADHD probands. Specifically, the distribution of 4R haplotypes did not differ between ADHD probands and controls, but there was an excess of 7R rare haplotypes in ADHD subjects (Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012). This finding was replicated a year later on a large sample (4 101 subjects) from the Pelotas Birth Cohort study (Tovo-Rodrigues, Rohde, Menezes, Polanczyk, Kieling, Genro, et al., 2013), again pointing to a possible role for not only rare allelic variants, but also for genetic heterogeneity in the aetiology of ADHD. This highlights the importance for future studies to not only focus on the number of repeats present in VNTR polymorphisms associated with ADHD, but to also look for DNA sequence variations within the repeat motifs.

### 2.7.5 Interactions between loci

Apart from allelic heterogeneity and rare genetic variants, another possible explanation for the mixed findings is the presence of interactions between loci influencing ADHD. Various studies have found interaction effects between two or more loci to influence ADHD (Carrasco, Rothhammer, Moraga, Henríquez, Chakraborty, Aboitiz, et al., 2006; Gabriela, John, Magdalena, Ariadna, Francisco, Liz, et al., 2009; Jain, Vélez, Acosta, Palacio, Balog, Roessler, et al., 2012; H. Kim, J.I. Kim, H. Kim, J.-W. Kim, & B.-N. Kim, 2017). In keeping with the dopamine hypothesis of ADHD, Carrasco, Rothhammer, Moraga, Henríquez, Chakraborty, Aboitiz, et al. (2006) found a significant interaction effect between homozygosity for the 10-repeat allele of the *DAT1* 3’ uVNTR, and heterozygosity for the 7-repeat allele of the *DRD4* exon III VNTR on ADHD. The co-occurrence of homozygosity for
the 10-repeat allele of the DAT1 3’ uVNTR and heterozygosity for the 7-repeat allele of the DRD4 exon III VNTR was significantly more frequent in ADHD cases than in their unaffected siblings. This finding was refuted by Gabriela, John, Magdalena, Ariadna, Francisco, Liz, et al. (2009) who found no significant interaction effect between these loci and ADHD. However, latter researchers did find that ADHD patients with internalised comorbidities (i.e. anxiety and depression) did have a lesser frequency of the 10/10 DAT1 genotype co-occurring with the 7/7 DRD4 genotype.

Apart from the interaction with the DRD4 gene, studies have also suggested that the 10-repeat allele of the DAT1 3’ uVNTR interacts with another locus within the DAT1 gene to influence ADHD (Asherson, Brookes, Franke, Chen, Gill, Ebstein, et al., 2007; Brookes, Xu, Anney, Franke, Zhou, Chen, et al., 2008). Specific haplotypes of the DAT1 gene containing the 10-repeat allele as one of its components shows strong associations with ADHD (Asherson, Brookes, Franke, Chen, Gill, Ebstein, et al., 2007; Barr, Xu, Kroft, Feng, Wigg, Zai, et al., 2001; Brookes, Xu, Chen, Zhou, Neale, Lowe, et al., 2006; Hawi, Kent, Hill, Anney, Brookes, Barry, et al., 2010), thus, supporting this hypothesis. More specifically, a haplotype consisting of the 10-repeat allele of the 3’ DAT1 VNTR, as well as a 6-repeat allele of a 30 base pair VNTR located in intron 8 of the same gene, has shown strong associations with ADHD in numerous studies (Asherson, Brookes, Franke, Chen, Gill, Ebstein, et al., 2007; Brookes, Mill, Guindalini, Curran, Xu, Knight, et al., 2006; Brookes, Xu, Chen, Zhou, Neale, Lowe, et al., 2006; Hawi, Kent, Hill, Anney, Brookes, Barry, et al., 2010). The 10-6 haplotype is one of four commonly occurring haplotype combinations of the two markers, with the other three combinations being 9-5, 9-6, and 10-5 (Asherson, Brookes, Franke, Chen, Gill, Ebstein, et al., 2007; Brookes, Mill, Guindalini, Curran, Xu, Knight, et al., 2006). The study by Brookes, Mill, Guindalini, Curran, Xu, Knight, et al. (2006) was done in both a Taiwanese and an English sample, with the 10-6 haplotype being associated with ADHD in both these samples. The diversity of these two samples led the researchers to speculate that this association might be generalisable to populations across the world. However, similar studies have not yet been conducted in African populations, and before this is done, no conclusions can be reached about the generalisability of the finding to African population groups.
2.7.6 The influence of the environment and gene-environment interactions

Another possible explanation for the mixed findings relates to the influence of environmental factors and the possible interactions between genetic and environmental factors. Banerjee, Middleton, and Faraone (2007) describe gene-environment interactions as “any phenotypic events that are due to interactions between the environment and genes”. Nigg, Nikolas, and Burt (2010) note that the high heritability found for ADHD may have misled researchers to not study gene-environment interactions as frequently as for other disorders. However, high heritability should actually prompt the study of gene-environment interactions, since the genetic proportion of variance in standard behavioural genetic analysis includes variance explained by gene-environment interactions (Nigg, Nikolas, & Burt, 2010).

Family studies can be used to determine whether the risk of a disorder is increased in individuals who are at a higher inferred genetic risk and exposed to an environmental risk, compared to individuals at lower genetic risk (Ficks, & Waldman, 2009; Milberger, Biederman, Faraone, Guite, & Tsuang, 1997). Prior to the use of specific genes to test for possible gene-environment interactions, family studies were used in this way to determine the degree of interaction between genes and the environment (Ficks, & Waldman, 2009; Milberger, Biederman, Faraone, Guite, & Tsuang, 1997). Banerjee, Middleton, and Faraone (2007) categorised possible environmental risk factors into biological and psychosocial adversity. In their review, Banerjee, Middleton, and Faraone (2007) named diet, toxins in the environment, pregnancy and delivery complications, and foetal exposure to alcohol and maternal smoking as possible biological risk factors for the development of ADHD. Both Oerlemans, Burmanje, Franke, Buitelaar, Hartman, and Rommelse (2016) and Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al. (2012) also note that environmental risk factors for ADHD seem to be particularly related to factors surrounding pregnancy and birth.

Milberger, Biederman, Faraone, Guite, and Tsuang (1997) hypothesised that if pregnancy, delivery and infancy complications (PDICs) and genetic factors interact to increase the risk of ADHD, an interaction term between PDICs and a variable representing genetic risk should be significant in a logistic regression model predicting ADHD status. The interaction term between PDICs and genetic risk (quantified as having at least one first-
degree relative with ADHD) was not significant in the prediction of ADHD status. The researchers did, however, find a positive association between PDICs and ADHD, thus supporting the findings by Sprich-Buckminster, Biederman, Milberger, Faraone, and Lehman (1993) that PDICs is a genetically independent environmental risk factor for ADHD.

No other similar studies looking at the moderating influence of familial factors on the influence of environmental factors on ADHD could be found. With the advancements in molecular technology, studies mostly started to focus on the interactions between specific genes and specific environmental factors (Brinksma, Hoekstra, Van den Hoofdakker, De Bildt, Buitelaar, Hartman, et al., 2017; Brookes, Mill, Guindalini, Curran, Xu, Knight, et al., 2006; Ficks, & Waldman, 2009; Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al., 2012; Neuman, Lobos, Reich, Henderson, Sun, & Todd, 2007). That said, as noted in section 2.7.3, recent studies have been conducted on the aetiological distinction between simplex and multiplex ADHD (Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al., 2014; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015; Oerlemans, Burmanje, Franke, Buitelaar, Hartman, & Rommelse, 2016). This concept is closely related to the concept of determining the role of gene-environment interactions through family studies. Oerlemans, Burmanje, Franke, Buitelaar, Hartman, and Rommelse (2016) conducted a study where they partitioned pregnancy and delivery complications into shared and non-shared environmental factors, and partitioned ADHD families into single-incidence (simplex) and multi-incidence (multiplex) families. They tested whether simplex and multiplex ADHD can be seen as aetiologically distinct, with simplex ADHD being influenced by non-shared environmental factors and multiplex ADHD by environmental factors shared between family members. They concluded that there were no pre- and perinatal aetiological differences between simplex and multiplex ADHD.

Studies, like the ones by Sprich-Buckminster, Biederman, Milberger, Faraone, and Lehman (1993), Milberger, Biederman, Faraone, Guite, and Tsuang (1997), and Oerlemans, Burmanje, Franke, Buitelaar, Hartman, and Rommelse (2016), can still be useful in countries such as South Africa with limited resources for conducting molecular genetic studies. These types of studies can serve as indicators pointing subsequent molecular genetic studies in the right direction, and thus prevent the wasting of resources.
2.7.6.1 Maternal stress during pregnancy

One factor that has been researched quite extensively is the effect of maternal stress during pregnancy on the developing fetus and later outcomes (Glover, 2014, 2015; Glover, Ahmed-Salim, & Capron, 2016; Ruiz, & Avant, 2005; Van den Bergh, Van den Heuvel, Lahti, Braeken, De Rooij, Entringer, et al., 2017).

Stress is defined as any factor that challenges the body’s ability to maintain homeostasis (Odendaal, Human, Groenewald, & Bavanisha, 2011; Sherwood, 2015). Odendaal, Human, Groenewald, and Bavanisha (2011) make it clear that this does not necessarily refer to an adverse situation. In line with this idea, Sherwood (2015) mentions that stress can be caused by a wide variety of stimuli, including physical, chemical, physiologic, or psychological factors. In the human body, stress results in a cascade of reactions starting with the stimulation of the hypothalamus to secrete corticotropin-releasing hormone (CRH). CRH in turn stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH). Finally, ACTH stimulates the adrenal cortex to secrete cortisol. Cortisol helps the body cope with stress in various ways. Overall, it increases the availability of the metabolic fuels and building blocks needed by the body to relieve stress. This includes an increase in blood glucose, an increase in blood amino acids, as well as an increase in blood fatty acids (Hobel, Goldstein, & Barrett, 2008; Odendaal, Human, Groenewald, & Bavanisha, 2011; Sherwood, 2015). Normally, a negative feedback control system is in place whereby an increase in cortisol inhibits the secretion of CRH by the hypothalamus and that of ACTH by the anterior pituitary. However, stress, and in particular chronic stress, can result in this negative feedback loop not functioning properly, and leading to dramatic increases in cortisol in the body (Hobel, Goldstein, & Barrett, 2008; Sherwood, 2015). Should this happen during the early phases of pregnancy (before 20 weeks), it may lead to the fetus being exposed to excessive amounts of cortisol. Usually the placenta protects the fetus from exposure to high levels of cortisol through the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) which metabolises glucocorticoids. However, during the early phases of pregnancy, 11β-HSD is not yet functional (Hobel, Goldstein, & Barrett, 2008; Seckl, Cleasby, & Nyirenda, 2000). In addition to the fetus being exposed to an excess amount of cortisol under stressful conditions, it has also been found that cortisol stimulates CRH gene expression in the placenta, resulting in a rise in CRH levels (Odendaal, Human, Groenewald, & Bavanisha, 2011; Sandman, Glynn,
Thus, contrary to the negative feedback effect of cortisol on CRH levels in non-pregnant women, cortisol actually increases CRH levels in pregnant women. This in turn stimulates ACTH and cortisol release (Challis, Sloboda, Matthews, Holloway, Alfaidy, Patel, et al., 2001; Hobel, Goldstein, & Barrett, 2008).

Apart from excessive exposure to cortisol, maternal stress may also influence the development of the fetus by means of fetal hypoxia. This happens as a result of decreased blood flow to the fetus due to the constriction of the uterine artery brought about by the vasoconstrictive effects of the corticosteroid and chatecolamine hormones secreted as part of the body’s response to stress (Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al., 2012; Mulder, Robles de Medina, Huizink, Van den Bergh, Buitelaar, & Visser, 2002; Teixeira, Fisk, & Glover, 1999).

Concerning ADHD in particular, a number of studies have found that maternal anxiety or excessive stress during pregnancy results in more severe ADHD symptoms. These studies spanned from children as young as two years old to children 12 years of age (Grizenko, Fortier, Gaudreau-Simard, Jolicoeur, & Joober, 2015; Grizenko, Shayan, Polotskaia, Ter-Stepanian, & Joober, 2008; Laucht, Esser, Baving, Gerhold, Hoesch, Ihle, et al., 2000; O’Connor, 2002; Rodriguez, & Bohlin, 2005; Ronald, Pennell, & Whitehouse, 2011; Van den Bergh, & Marcoen, 2004). However, the effect of stress on severity of ADHD symptoms differed across the studies. For example, Van den Bergh, and Marcoen (2004) found that maternal trait anxiety during pregnancy explained as much as 22% of the variance in ADHD symptoms. In contrast, Ronald, Pennell, and Whitehouse (2011) reported only a small association between stressful life events during pregnancy and ADHD behaviours. These findings may reflect the influence of the different measuring instruments and constructs used to assess and define the various stressors. Another possible explanation for the discrepancy in findings is the different variables being controlled for in the different studies. For example, in the Ronald, Pennell, and Whitehouse (2011) study, controlled variables included maternal age at conception and maternal alcohol consumption. Whereas in the Van den Bergh, and Marcoen (2004) study, factors such as postnatal maternal anxiety and smoking during pregnancy were controlled. However, it is also possible that the association between maternal prenatal stress and ADHD is moderated by genetic factors, resulting in
mixed findings due to genotype frequency differences between the sample groups. Thus, so-called gene-environment interactions may be at play.

A limited number of studies have looked specifically at gene-environment interactions between prenatal maternal stress during pregnancy and ADHD symptoms in children. The first study doing this was by Grizenko and colleagues (2012). In this study, stress during pregnancy was operationally defined as stressful life events experienced during pregnancy. The researchers investigated whether alleles of the DRD4 exon III VNTR and the DAT1 3’ uVNTR significantly interacted with maternal stress in influencing the severity of ADHD symptomology (Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al., 2012). According to a review by Ficks, and Waldman (2009), these two polymorphisms are the ones most frequently studied for possible interaction with environmental factors in relation to ADHD. Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al. (2012) found main effects for both maternal stress during pregnancy and the DRD4 exon III VNTR. In addition, the researchers found a possible interaction effect between the 7/7 genotype of the DRD4 exon III VNTR and maternal stress during pregnancy. The most severe symptoms of ADHD were found in children who were both exposed to maternal prenatal stress and carried the 7/7 genotype of the DRD4 exon III VNTR.

Subsequent to the Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al. (2012) study, Choudhry, Sengupta, Grizenko, Fortier, Thakur, Bellingham, et al. (2012) found highly significant interaction effects between SNPs in the LPHN3 gene and maternal stress in relation to ADHD symptomology. Maternal stress was given the same operational definition as in the Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al. (2012) study. Focusing on maternal prenatal anxiety, O’Donnell, Glover, J. Lahti, M. Lahti, Edgar, Räikkönen, et al. (2017) found a significant gene-environment interaction effect between maternal prenatal anxiety and the Val158Met polymorphism in the COMT gene on child ADHD symptoms. In two large cohort groups, maternal prenatal anxiety had a greater impact on ADHD symptoms in children homozygous for the Val allele than in children either carrying the Val/Met or the Met/Met genotypes. Although replication studies are needed, gene-environment interactions involving maternal stress seems like a promising line of enquiry in deciphering the aetiology of ADHD.
2.7.6.2 Maternal smoking during pregnancy

A number of studies have found statistically significant associations between maternal smoking during pregnancy and ADHD (Gustafsson, & Källén, 2011; He, Chen, Zhu, Hua, & Ke, 2017; Kovess, Keyes, Hamilton, Pez, Bitfoi, Koç et al., 2015; Linnet, Dalsgaard, Obel, Wisborg, Henriksen, Rodriguez, et al., 2003; Mick, Biederman, Faraone, Sayer, & Kleinman, 2002; Nomura, Marks, & Halperin, 2010; Thapar, Fowler, Rice, Scourfield, Van den Bree, Thomas, et al., 2003; Zhu, Olsen, Liew, Li, Niclasen, & Obel, 2014). However, as noted by Skoglund, Chen, D′Onofrio, Lichtenstein, and Larsson (2014) and Gustavson, Ystrom, Stoltenberg, Susser, Surén, Magnus, et al. (2017), the mechanisms through which maternal smoking influences ADHD are poorly understood. Several recent studies have found that maternal smoking during pregnancy does not cause ADHD through a direct intrauterine effect. Rather, these studies found that the relationship between maternal prenatal smoking and ADHD are due to unmeasured familial confounding. This includes both genetic and household-level factors (Gustavson, Ystrom, Stoltenberg, Susser, Surén, Magnus, et al., 2017; Langley, Heron, Smith, & Thapar, 2012; Lindblad, & Hjern, 2010; Obel, Zhu, Olsen, Breining, Grønborg, Gissler, et al., 2016; Skoglund, Chen, D′Onofrio, Lichtenstein, & Larsson, 2014; Thapar, Rice, Hay, Boivin, Langley, Van den Bree, et al., 2009). In contrast to these findings, a number of studies found that maternal smoking during pregnancy accounted for a significant percentage of the variance in ADHD symptoms, even after controlling for familial risk (Knopik, Marceau, Bidwell, Palmer, Smith, Todorov, et al., 2016; Mick, Biederman, Faraone, Sayer, & Kleinman, 2002; Nomura, Marks, & Halperin, 2010; Thapar, Fowler, Rice, Scourfield, Van den Bree, Thomas, et al., 2003). In this regard, Nomura, Marks, and Halperin (2010) noted that these findings point to a causal link between in utero exposure to cigarette smoke and the occurrence of ADHD.

Although the above findings are contradictory, a number of possible biological mechanisms have been proposed as causal links between cigarette smoking during pregnancy and the occurrence of ADHD (Banerjee, Middleton, & Faraone, 2007; Blood-Siegfried, & Rende, 2010; Ernst, Moolchan, & Robinson, 2001). The dopaminergic system of an animal model that had been exposed to nicotine prenatally has been found to be hypoactive and hyporesponsive when exposed to exogenous stimulation (Banerjee, Middleton, & Faraone, 2007; Slotkin, 1998). This is in line with the dopamine hypothesis of
ADHD that stipulates that ADHD results from hypodopaminergic synapses. In addition, it has been found that binding of nicotine to neural nicotine acetylcholine receptors results in increased dopamine release in striatal and cortical regions of the brain (Cao, Surowy, & Puttfarcken, 2005; Neuman, Lobos, Reich, Henderson, Sun, & Todd, 2007). This can possibly also influence behaviour. However, more studies are needed to determine whether smoking during pregnancy really does add significantly to the variance observed in ADHD, over and above the influence of unmeasured familial confounding.

Another biological mechanism proposed by Todd, and Neuman (2007) opens up the possibility for gene-environment interactions between cigarette smoking during pregnancy and the dopamine receptor and transporter genes. These researchers propose that the nicotine stimulates pre-synaptic high affinity α4β2 neuronal nicotinic receptor complexes in the fetus. This results in an increase in the levels of dopamine released by the developing dopaminergic neurons, which in turn results in changes in neurite outgrowth and branching post-synaptically. However, the magnitude of this morphogenic effect is mediated by presynaptic dopamine transporter number and function, as well as post-synaptic dopamine receptor number and function. Both the dopamine transporters (e.g. DAT1) and receptors (e.g. DRD4) are proteins coded for by genes and affected by genetic variations (Todd, & Neuman, 2007). Thus, it is possible that maternal cigarette smoking during pregnancy interacts with the dopamine transporter and receptor genes to influence ADHD symptoms and severity. Results of studies looking for possible interaction effects between dopamine genes and cigarette smoking have, however, been mixed. A study by Neuman, Lobos, Reich, Henderson, Sun, and Todd (2007) found a significant interaction effect between the 7-repeat allele of the DRD4 exon III VNTR and cigarette smoking. Children who carried at least one DRD4 7-repeat allele and whose mothers smoked during pregnancy were at the highest risk of being diagnosed with any of the ADHD subtypes. However, a later study by Altink, Arias-Vásquez, Franke, Slaats-Willemse, Buschgens, Rommelse, et al. (2008) found no significant interaction effect between the DRD4 7-repeat allele and cigarette smoking. The latter finding was replicated in a recent study by Van der Meer, Hartman, Van Rooij, Franke, Heslenfeld, Oosterlaan, et al. (2017), with the researchers finding no significant interaction effect between the DRD4 exon III VNTR and prenatal exposure to smoking on ADHD severity.
For the DAT1 gene, Neuman, Lobos, Reich, Henderson, Sun, and Todd (2007) found a significant interaction effect between a 440 base pair allele of the DAT1 3’ uVNTR polymorphism and maternal cigarette smoking. The risk of diagnosis for any form of ADHD was greatest when the mothers smoked during pregnancy and the child’s genotype included the DAT1 440 base pair allele. Significantly higher hyperactive-impulsive symptom scores were observed in children exposed to prenatal smoking and who were homozygous for the 480 base pair allele of the DAT1 3’ uVNTR (Kahn, Khoury, Nichols, & Lanphear, 2003; Becker, El-Faddagh, Schmidt, Esser, & Laucht 2008). It should be noted that in the Becker, El-Faddagh, Schmidt, Esser, and Laucht (2008) study, this effect was restricted to males. In contrast to these positive findings, studies by both Kieling, Hutz, Genro, Polanczyk, Anselmi, Camey, et al. (2013) and Van der Meer, Hartman, Van Rooij, Franke, Heslenfeld, Oosterlaan, et al. (2017) failed to find a significant gene by environment interaction effect between prenatal exposure to smoking and the DAT1 gene on ADHD symptom severity. Clearly, more studies are needed to elucidate the exact nature of the interaction between dopamine genes and prenatal cigarette smoking in influencing ADHD.

2.7.6.3 Maternal alcohol use during pregnancy

From the literature, there seems to be a definite link between prenatal alcohol exposure and symptoms of ADHD in offspring (Coles, Platzman, Lynch, & Freides, 2002; Eilertsen, Gjerde, Reichborn-Kjennerud, Ørstavik, Knudsen, Stoltenberg, et al., 2017; Fryer, McGee, Matt, Riley, & Mattson, 2007; Infante, Moore, Nguyen, Fouligas, Mattson, & Riley, 2015; Lee, Mattson, & Riley, 2004; Mattson, Calarco, & Lang, 2006). That said, two reviews have reported that the results across studies on the effect of alcohol exposure on ADHD symptoms are inconsistent (Banerjee, Middleton, & Faraone, 2007; Linnet, Dalsgaard, Obel, Wisborg, Henriksen, Rodriguez, et al., 2003). For example, in the review by Linnet, Dalsgaard, Obel, Wisborg, Henriksen, Rodriguez, et al. (2003), the researchers noted that only half of the papers included in the review reported a significant effect for prenatal alcohol exposure on inattention and impulsivity. In addition, in a recent meta-analysis, Flak, Su, Bertrand, Denny, Kesmodel, and Cogswell (2014) could not find any significant association between prenatal alcohol exposure and neuropsychological outcomes in children, including inattention. Furthermore, some studies have found behavioural and neurocognitive differences, as well as differences in executive functioning between children with ADHD and children affected by
prenatal alcohol exposure (Coles, Platzman, Raskind-Hood, Brown, Falek, & Smith, 1997; Crocker, Vaurio, Riley, & Mattson, 2009, 2011; Kingdon, Cardoso, & McGrath, 2016; Vaurio, Riley, & Mattson, 2008). These results suggest that the ADHD-like symptoms in children prenatally exposed to alcohol may be aetio logically different from that in children with only a diagnosis of ADHD and no prenatal alcohol exposure.

However, numerous studies have found a significant association between prenatal alcohol exposure and higher rates of ADHD diagnosis or ADHD symptoms, and should not be ignored (Bhatara, Loudenberg, & Ellis, 2006; Eilertsen, Gjerde, Reichborn-Kjennerud, Ørstavik, Knudsen, Stoltenberg, et al., 2017; Fryer, McGee, Matt, Riley, & Mattson, 2007; Han, Kwon, Ha, Paik, Lim, Guy Lee, et al., 2015; Infante, Moore, Nguyen, Fourligas, Mattson, & Riley, 2015; Knopik, Sparrow, Madden, Bucholz, Hudziak, Reich, et al., 2005; Mick, Biederman, Faraone, Sayer, & Kleinman, 2002). For instance, Han, Kwon, Ha, Paik, Lim, Guy Lee, et al. (2015) found that children who were exposed to alcohol prenatally have a 1.55 times increased risk of being diagnosed with ADHD compared to children not exposed. In addition, attention deficits and impulsivity symptoms (Atalar, Uzbay, & Karakaş, 2016; Hausknecht, Acheson, Farrar, Kieres, Shen, Richards, et al., 2005), as well as hyperactivity (Muñoz-Villegas, Rodríguez, Giordano, & Juárez, 2017) have also been shown in rat models prenatally exposed to ethanol.

Various factors influence whether alcohol exposure in utero will have adverse effects, as well as the degree of these effects (Bhatara, Loudenberg, & Ellis, 2006). These factors include the timing of exposure during pregnancy and the amounts of alcohol consumed by the mother. These researchers also caution that environmental and biological factors, as well as the interactions between the various factors, may influence the effects of prenatal alcohol exposure (Bhatara, Loudenberg, & Ellis, 2006). Concerning possible biological mechanisms, Goodlett, and Horn (2001) propose that alcohol can exert negative effects on the developing central nervous system of the fetus, either directly or indirectly. Directly, alcohol can have negative effects on the fetal tissue, whereas indirectly, alcohol can interfere with the maternal structures supporting the growing fetus. Alfonso-Loeches, and Guerri (2011) note in a review article that alcohol interferes with various molecular, biochemical, and cellular processes that are crucial for the correct formation of the developing central nervous system. Apart from proven negative consequences of alcohol exposure on fetal cell
proliferation, migration, growth and differentiation (Alfonso-Loeches, & Guerri, 2011), it has also been found that alcohol exposure during pregnancy can result in damage to the prefrontal cortex (Guerri, Bazinet, & Riley, 2009; Hamilton, Hernandez, Krebs, Bucko, & Rhodes, 2017; Sowell, Thompson, Mattson, Tessner, Jernigan, Riley, et al., 2002). This is of note since the prefrontal cortex is known to be important in the regulation of attentional processes (Asplund, Todd, Snyder, & Marois, 2010; Bichot, Heard, DeGennaro, & Desimone, 2015; Schafer, & Moore, 2011). Furthermore, ethanol has been found to have an influence on the migration of nerve cells and to interfere with neurochemical hormone production. Both of these factors may influence behaviour (Linnet, Dalsgaard, Obel, Wisborg, Henriksen, Rodriguez, et al., 2003; Pratt, 1984).

A limited number of studies have been conducted on the role of gene-environment interactions between genes in the dopamine system and prenatal alcohol exposure on ADHD. In a review by Ficks, and Waldman (2009) on studies examining the influence of gene-environment interactions in ADHD, no studies found a significant interaction effect between prenatal alcohol exposure and the DRD4 exon III VNTR. Concerning the DAT1 3’ uVNTR, Ficks, and Waldman (2009) also found no evidence of interaction for this polymorphism with prenatal alcohol use on ADHD symptoms in any of the studies reviewed. This lack of a significant interaction effect between either the DRD4 exon III VNTR or the DAT1 3’ uVNTR, and prenatal alcohol exposure on ADHD was replicated recently in a study by Van der Meer et al. (2017). However, Brookes, Mill, Guindalini, Curran, Xu, Knight, et al. (2006) found that a common haplotype in the DAT1 gene interacted with maternal alcohol use during pregnancy to influence ADHD in a Taiwanese and an English sample. The haplotype in question consisted of the combination of the 10-repeat allele of the DAT1 3’ uVNTR and the 3-repeat allele of an intron 8 VNTR in the same gene. Latter VNTR consists of a 30 base pair repeat sequence repeated a variable number of times (Brookes, Mill, Guindalini, Curran, Xu, Knight, et al., 2006). This finding highlights the importance of not only looking at common polymorphisms in candidate genes in isolation when looking at gene-environment interactions in ADHD.
2.7.6.4 Parental age and ADHD

Findings regarding parental (both maternal and paternal) age at the birth of offspring and ADHD have been conflicting. For paternal age, Shimada, Kitamoto, Todokoro, Ishii-Takahashi, Kuwabara, Kim, et al. (2012) and D’Onofrio, Rickert, Frans, Kuja-Halkola, Almqvist, Sjölander, et al. (2014) found that higher paternal age was a risk factor for ADHD. D’Onofrio, Rickert, Frans, Kuja-Halkola, Almqvist, Sjölander, et al. (2014) noted that this finding supported the hypothesis that mutations during spermatogenesis in older fathers increased risk for ADHD. In contrast to this hypothesis, however, studies by Chudal, Joelsson, Gyllenberg, Lehti, Leivonen, Hinkka-Yli-Salomäki, et al. (2015), Ghanizadeh (2014) and Oerlemans, Burmanje, Franke, Buitelaar, Hartman, and Rommelse (2016) all showed a higher risk for ADHD in offspring from younger fathers compared to older fathers.

Concerning the effect of maternal age at child birth on risk for ADHD, Shimada, Kitamoto, Todokoro, Ishii-Takahashi, Kuwabara, Kim, et al. (2012) found higher maternal age in persons with ADHD. Partially replicating this finding, Ghanizadeh (2014) found advanced maternal age to be associated with greater hyperactivity/impulsivity symptom severity, but not inattention symptom severity, in children with ADHD. In contrast to these findings, Chudal, Joelsson, Gyllenberg, Lehti, Leivonen, Hinkka-Yli-Salomäki, et al. (2015), Chang, Lichtenstein, D’Onofrio, Almqvist, Kuja-Halkola, Sjölander, et al. (2014), Gustafsson and Källén (2011), Valdimarsdóttir, Hrafnsdóttir, Magnússon, and Gudmundsson (2006), and Oerlemans et al. (2016) all found that young maternal age was associated with ADHD. Particularly salient is the finding by Chang, Lichtenstein, D’Onofrio, Almqvist, Kuja-Halkola, Sjölander, et al. (2014) that teenage childbirth (mothers younger than 20 years) resulted in the risk for ADHD increasing by 78%. These researchers attributed much of this increased risk to genetic factors transmitted from mothers to offspring that increased the risk for both ADHD and young maternal age.

Further confusing the picture, a study by Gabis, Raz, and Kesner-Baruch (2010) found that paternal age at the child’s birth had no effect on the development of ADHD. Similarly, Amiri, Malek, Sadegfard, and Abdi (2012) found no difference in either maternal or paternal age at child birth between an experimental ADHD group and a reference control group. Clearly, more research is needed to clarify the issue of parental age and ADHD.
Thus far, according to the current researcher’s knowledge, no studies have been conducted on the effects of interactions between parental age and specific polymorphisms on ADHD.

2.7.6.5 Preterm birth and children born small for gestational age

Many studies found that both children born preterm and children born small for gestational age are at increased risk for developing ADHD or symptoms of ADHD (Aarnoudse-Moens, Weisglas-Kuperus, Van Goudoever, & Oosterlaan, 2009; Bhutta, Cleves, Casey, Cradock, & Anand, 2002; Johnson, & Marlow, 2011; Lindstrom, Lindblad, & Hjern, 2011; Linnet, 2006; Mathewson, Chow, Dobson, Pope, Schmidt, & Van Lieshout, 2017; Murray, Pearson, Fernandes, Santos, Barros, Victora, et al., 2016; Pettersson, Sjölander, Almqvist, Anckarsäter, D’Onofrio, Lichtenstein et al., 2015; Singh, Kenney, Ghandour, Kogan, & Lu, 2013; Sucksdorff, Lehtonen, Chudal, Suominen, Joelsson, Gissler, et al., 2015; Van Baar, Vermaas, Knots, De Kleine, & Soons, 2009). A review by Johnson and Marlow (2011), pooling results from existing reports, indicates that very prematurely born children/children with very low birth weight have a two-to three-fold increased risk for ADHD. In addition, children born extremely preterm or with extremely low birth weights showed a four-fold increased risk for ADHD. This result was replicated in a meta-analysis by Mathewson, Chow, Dobson, Pope, Schmidt, and Van Lieshout (2017). This meta-analysis was conducted on 41 studies published between January 1990 and May 2016. Comparisons were drawn between children born at extremely low birth weight (less than 1 000g at birth) and normal controls on risk for inattention and hyperactivity. Results showed a significantly greater risk for children to show symptoms of both inattention and hyperactivity if they were born at extremely low birth weights. It has also been shown that children born moderately preterm, late preterm, early term, or children born at term with moderately low birth weights, also show more ADHD characteristics than children born at term with normal birth weights (Lindstrom, Lindblad, & Hjern, 2011; Linnet, 2006; Sucksdorff, Lehtonen, Chudal, Suominen, Joelsson, Gissler, et al., 2015; Van Baar, Vermaas, Knots, De Kleine, & Soons, 2009).

In a study by Groen-Blokhuis, Middeldorp, Van Beijsterveldt, and Boomsma (2011), the researchers found that the effect of low birth weight on attention problems cannot be ascribed to socioeconomic status, or tobacco or alcohol consumption during pregnancy. The
study by Lindstrom, Lindblad, and Hjern (2011) also found that the effects of preterm birth on increased risk for ADHD cannot be explained by genetic, perinatal, or socioeconomic confounding, pointing to a possible causal association between low birth weight/preterm birth and the presence of ADHD symptomology. In addition, Murray, Pearson, Fernandes, Santos, Barros, Victoria, et al. (2016) conducted a study comparing cohorts from a high-income country and a middle-income country to determine whether low birth weight and small size for gestational age play a causal role in ADHD diagnosis and ADHD symptoms. These researchers found a possible causal role for fetal growth impairment on the occurrence of attention difficulties, but not full ADHD diagnosis, in childhood. Groen-Blokhuis, Middeldorp, Van Beijsterveldt, and Boomsma (2011) hypothesised that attention problems are caused by impaired neurodevelopment due to deficient nourishment in utero. Along similar lines, Johnson, and Marlow (2011) postulate that the association between premature birth/low birth weight and the inattentive subtype of ADHD can be explained by impairment in normal brain growth and maturation. Latter authors caution, however, that further research is needed to clarify the aetiological links.

Surprisingly, very few studies have thus far been conducted that tested for interaction effects between specific dopaminergic genes and low birth weight/preterm birth on ADHD. Only three studies could be found in this regard (Brinksma, Hoekstra, Van den Hoofdakker, De Bildt, Buitelaar, Hartman, et al., 2017; Jackson, & Beaver, 2015; Langley, Turic, Rice, Holmans, Van den Bree, Craddock, et al., 2008). The first, conducted by Langley, Turic, Rice, Holmans, Van den Bree, Craddock, et al. (2008), did not find any significant gene by environment interactions between any of four dopaminergic genes and birth weight on ADHD diagnosis. The genes and polymorphisms looked at included the DAT1 3’ uVNTR, the DRD4 exon III VNTR, and the DRD5 (CA)n microsatellite. The study by Jackson, and Beaver (2015), in contrast, found significant interaction effects between birth weight and both the DAT1 3’ uVNTR and the DRD4 exon III VNTR, as well as the DRD2 Taq1A polymorphism, on ADHD symptomology. For the DAT1 3’ uVNTR, the 10-repeat allele was viewed as the risk allele, whilst the A1 allele of the DRD2 polymorphism and the 7 to 10 repeat alleles of the DRD4 polymorphism, were viewed as risk alleles. The researchers made use of a sibling-design and found that carrying any of these risk alleles resulted in a stronger association between low birth weight and ADHD symptomology. Finally, the most recent study
conducted by Brinksma, Hoekstra, Van den Hoofdakker, De Bildt, Buitelaar, Hartman, et al. (2017) did not find any significant gene by environment interaction effects between low birth weight and the DRD2 or DRD4 polymorphisms on ADHD; nor between low birth weight and the COMT Val158Met polymorphism on ADHD. Latter researchers did, however, find a significant interaction effect between the MAOA-uVNTR promotor polymorphism and low birth weight on ADHD. Individuals in their early adolescence who had low birth weight and who carried the low activity MAOA-uVNTR alleles were at greatest risk for enhanced ADHD severity, compared to all other adolescents in the study (Brinksma, Hoekstra, Van den Hoofdakker, De Bildt, Buitelaar, Hartman, et al., 2017).

In contrast to the positive associations in the above studies, twin studies have shown that environmental factors, rather than genes, mediate the relationship between low birth weight and ADHD (Hultman, Torrång, Tuvblad, Cnattingius, Larsson, & Lichtenstein, 2007; Lehn, Derks, Hudziak, Heutink, Van Beijsterveldt, & Boomsma, 2007; Thapar, Harold, Rice, Langley, & O’Donovan, 2007). Due to the identical nature of the genomes of monozygotic twins, any differences between them in behaviour must be caused by the effects of the non-shared environment and not mediated by genetic factors (Asbury, Dunn, & Plomin, 2006; Lehn, Derks, Hudziak, Heutink, Van Beijsterveldt, & Boomsma, 2007). Thus, findings of discordance in behaviours between monozygotic twins preclude the involvement of gene-environment interactions. In a study comparing monozygotic twins conducted by Asbury, Dunn, and Plomin (2006), the researchers found that members of twin pairs who were heavier at birth displayed less hyperactivity at age 7. In addition, a study on monozygotic twins conducted by Lehn, Derks, Hudziak, Heutink, Van Beijsterveldt, and Boomsma (2007) showed that members of twin pairs affected with ADHD had lower birth weights than their non-affected siblings. Similar results were reported by Hultman, Torrång, Tuvblad, Cnattingius, Larsson, and Lichtenstein (2007) who found significantly higher ADHD scores in monozygotic twin members who were lighter at birth. These findings point to the association between birth weight and ADHD being mediated by environmental factors, and not genetic factors.
2.7.6.6 Other obstetrical complications and ADHD

Apart from the afore-mentioned factors, other obstetrical complications have also been found to influence ADHD. These, in particular, include perinatal hypoxic-ischemic conditions such as preeclampsia, Apgar score below seven at five minutes, breech or transverse presentation, fetal distress, fetal post-maturity, duration of labour, and prolapsed/nuchal cord (Banerjee, Middleton, & Faraone, 2007; Zhu, Gan, Huang, Li, Qu, & Mu, 2016).

That said, a number of studies have failed to find a positive association between certain obstetrical complications and ADHD (Ketzer, Gallois, Martinez, Rohde, & Schmitz, 2012; Sauver, Barbaresi, Katusic, Colligan, Weaver, & Jacobsen, 2004; Wagner, Schmidt, Lemery-Chalfant, Leavitt, & Goldsmith, 2009). In a sample of 748 twins, Wagner, Schmidt, Lemery-Chalfant, Leavitt, and Goldsmith (2009) assessed a comprehensive range of mother- and child-specific obstetrical complications. These included factors such as maternal parity and gravidity, placental information, and fetal position at birth. Latter researchers found no strong or even moderate associations between these indices and ADHD symptoms in middle childhood. It should be noted that this does not necessarily mean that these obstetrical complications did not have an effect that diminished with time. Sauver, Barbaresi, Katusic, Colligan, Weaver, and Jacobsen (2004) also did not find any association between induction or augmentation of labour, mode of delivery, any operative procedures, pregnancy induced hypertension, and gestational diabetes and ADHD status in a case-control study of children with ADHD. Similarly, Ketzer, Gallois, Martinez, Rohde, and Schmitz (2012) did not find any significant differences in the number of caesarean deliveries, bad fetal positions, anaesthesia, preeclampsia/eclampsia, and placental abnormalities between cases with ADHD-inattentive type and unaffected controls.

In a study by Buschgens, Swinkels, Van Aken, Ormel, Verhulst, and Buitelaar (2009), the researchers created a composite index of pregnancy and delivery complications consisting of pregnancy complications, complicated deliveries, and hospitalisation of the mother or child. The pregnancy complications consisted of physical, social or psychological problems during pregnancy. The complicated deliveries included breech presentation and caesarean section. In contrast to the above findings, these researchers found that the number of pregnancy and delivery complications was directly related to parent and teacher reports of inattention in
children. Thus, children with a higher number of pregnancy and delivery complications obtained higher inattention scores. It thus seems that a combination of different pregnancy and delivery complications has a bigger influence on symptoms of ADHD than single predictors used separately. In the same study by Buschgens, Swinkels, Van Aken, Ormel, Verhulst, and Buitelaar (2009), no significant interaction effect was found between an index of familial risk and the index of pregnancy and delivery complications on inattention and hyperactivity/impulsivity scores. These results replicate findings by Milberger, Biederman, Faraone, Guite, and Tsuang (1997). Latter researchers also found a positive association between pregnancy and delivery complications and ADHD, but no significant interaction effect between pregnancy and delivery complications and quantitative genetic factors on ADHD.

Based on the Buschgens, Swinkels, Van Aken, Ormel, Verhulst, and Buitelaar (2009) study, a recent study by Brinksma, Hoekstra, Van den Hoofdakker, De Bildt, Buitelaar, Hartman, et al. (2017) created an index score for pregnancy (for example, physical, social or psychological problems during pregnancy), delivery (for example, breech presentation and caesarean section), and neonatal (for example, lack of oxygen, blood transfusion, jaundice) complications based on a total of 31 possible complications. However, instead of using an index of familial risk, as was done by Buschgens, Swinkels, Van Aken, Ormel, Verhulst, and Buitelaar (2009), Brinksma, Hoekstra, Van den Hoofdakker, De Bildt, Buitelaar, Hartman, et al. (2017) tested for an interaction effect between polymorphisms in actual candidate genes and the pregnancy, delivery, and neonatal complications index on ADHD. Included in the study were polymorphisms in four genes involved in the dopaminergic system, namely the DRD4 exon III VNTR, the Val158Met polymorphism in the COMT gene, the MAOA-uVNTR promoter polymorphism, and the DRD2 Taq1A polymorphism. In addition, a polymorphism in the gene encoding the serotonin transporter (the SLC6A4 gene), referred to as the serotonin-transporter-linked-polymorphic region, or 5-HTTLPR for short, was also included in the study. The 5-HTTLPR consists of a 44 base pair insertion/deletion that gives rise to two possible alleles, a short variant (S allele) and a long variant (L allele) (Heils, Teufel, Petri, Stöber, Riederer, Bengel, et al., 1996). Brinksma, Hoekstra, Van den Hoofdakker, De Bildt, Buitelaar, Hartman, et al. (2017) detected no significant gene by environment interaction effects for any of the genes linked to the dopaminergic system (DRD4, COMT, MAOA, and
DRD2). A significant interaction effect was detected for the 5-HTTLPR. Individuals homozygous for the L allele who had a greater number of pregnancy and delivery complications on the index showed more ADHD symptoms during early adolescence.

2.7.6.7 The role of epigenetics in gene-environment interactions in ADHD

The mechanisms through which environmental factors interact with genes to influence ADHD is not well understood, but evidence is emerging that epigenetic processes may be involved (Gervin, Nordeng, Ystrom, Reichborn-Kjennerud, & Lyle, 2017; Van Mil, Steegers-Theunissen, Bouwland-Both, Verbiest, Rijlaardsdam, Hofman, et al., 2014; Walton, Pingault, Cecil, Gaunt, Relton, Mill, et al., 2017; Xu, Chen, Luo, Tang, Zhang, Wu, et al., 2015). Epigenetics refers to changes in phenotype or gene expression that are inherited but are not caused by changes in the basic structure of the underlying DNA sequence. Rather, it involves modifications of the activation of certain genes, mediated by DNA methylation, physical changes to chromatin structure, and the action of siRNA molecules (Archer, Oscar-Berman, & Blum, 2011; Henikoff, & Matzke, 1997).

Regarding the biological mechanisms through which pre- and peri-natal environmental insults interact with genes to influence ADHD, evidence is accumulating that factors in the pre- and peri-natal environment disrupt normal brain develop with lasting effects on brain function and behaviour through epigenetic mechanisms (Bale, Baram, Brown, Goldstein, Insel, McCarthy, et al., 2010; Kundakovic, & Jaric, 2017; Weinhold, 2012). In fact, epigenetic disruption is particularly common during the prenatal period, with the epigenome being highly susceptible to environmental insults during early prenatal development (Jirtle, & Skinner, 2007; Kundakovic, & Jaric, 2017).

For example, individuals prenatally exposed to malnutrition and maternal stress due to famine during the Dutch Hunger Winter showed less DNA methylation of the IGF2 gene compared to their unexposed siblings (Heijmans, Tobi, Stein, Putter, Blauw, Susser, et al., 2008). In addition, prenatal exposure to maternal depression has been shown to be associated with increased methylation of the NR3C1 gene and decreased methylation of the BDNF gene (Braithwaite, Kundakovic, Ramchandani, Murphy, & Champagne, 2015; Oberlander, Weinberg, Papsdorf, Grunau, Misri, & Devlin, 2008). Furthermore, in a study by Toledo-Rodriguez, Lotfiipour, Leonard, Perron, Richer, Veillette, et al. (2010) an association
between prenatal exposure to maternal cigarette smoking and higher rates of DNA methylation of the \textit{BDNF} gene was shown. The authors suggested that prenatal exposure to maternal smoking may consequently lead to the down-regulation of \textit{BDNF} expression, resulting in modifications in the development and plasticity of the brain in utero.

The above findings lend support to the hypothesis that prenatal maternal insults can alter epigenetic programming in utero, influence the development and plasticity of the brain, and contribute to neurodevelopmental and behavioural deficits in offspring (Kundakovic, & Jaric, 2017).

\textbf{2.8 Conclusion}

From the above discussion, it should be clear that ADHD is a highly prevalent disorder, with substantial morbidity associated with its occurrence for both affected individuals and their families. Although great strides have been made in treating ADHD, there is still room for improvement. Determining the aetiology of a disorder is, however, paramount to the development of better treatment strategies.

Regarding the aetiology of ADHD, the disorder has been shown to be highly heritable, with genetic factors making a substantial contribution in the observed variance. However, the above discussion also makes it clear that studies into the molecular genetic architecture of ADHD have produced a myriad of conflicting findings. Resolving the conflicting findings is important so that definitive conclusions can be drawn regarding the genetic factors involved in the disorder. Numerous possible reasons for the high rates of conflicting findings were reviewed in the above discussion. This discussion focused on the possible heterogenous nature of ADHD, which could result in heterogeneous study populations and consequent conflicting findings if this heterogeneity is not taken into account.

Factors reviewed that could result in heterogenous samples included the aetiological nature of ADHD subtypes, the aetiological nature of ADHD comorbid with ODD, the aetiological nature of simplex versus multiplex ADHD, rare genetic variants in ADHD, the role of gene-gene interactions, and the role of gene-environment interactions in ADHD. Although previous studies have been conducted looking into each of these possibilities, no studies of this nature have been conducted with the South African population. The aim of the current
study was therefore to investigate the plausibility of each of these possible reasons for sample heterogeneity, and consequently conflicting findings, in a sample from South Africa. The findings from this study can provide methodological tools for future researchers to reduce sample heterogeneity in studies on the aetiology of ADHD. More homogeneous samples should in turn result in a reduction in the conflicted findings. This, in turn, should lead to an advancement into the understanding of the aetiology of ADHD, which could consequently lead to an improvement in the available treatment strategies.
Chapter 3

Sample Layout

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3.1 Sample layout

To place the following chapters in the broader context of the original sample gathered, a breakdown of the sampling process, along with the exclusion criteria applicable to each chapter and the resultant number of participants in each chapter, are presented here. Participants were recruited through social media pages via the Attention Deficit and Hyperactivity Support Group of Southern Africa (ADHASA), as well as through medical professionals working with children diagnosed with ADHD in the Bloemfontein area, Free State province, South Africa. The recruitment process targeted parents of children between the ages of 5 and 18 previously diagnosed with ADHD by a healthcare professional, and asked parents who were willing to participate to complete questionnaires for themselves and all of their children, including the ones not previously diagnosed with ADHD. Due to the length of the questionnaires and the fact that it had to be completed for all siblings in a nuclear family, many parents opted to only complete the questionnaire for their children, and not for themselves, resulting in a dearth of data regarding parental ADHD. The exact nature of the questionnaires is covered in each of the chapters.

The original sample consisted of 162 nuclear families, with data available for 291 children, 67 mothers, and 44 fathers. Thus, the full sample consisted of 402 participants for who data was available, from 162 nuclear families. Of the 291 children (all between the ages of 5 and 18), 192 (66.0%) were male, and 92 (31.6%) were female, whilst for 7 children (2.4%) gender was not indicated. Regarding ADHD diagnosis by a healthcare professional as reported by the parents, 191 (65.6%) of the children were reported as having been diagnosed with ADHD, while the remaining 100 children (34.4%) had never before received a diagnosis of ADHD and were therefore seen as not affected by the disorder.

In chapter 4 (Familial aggregation of Attention-Deficit/Hyperactivity Disorder (ADHD) subtypes in a South African sample), only nuclear families where at least one parent completed the questionnaire for themselves and for their children were included. Adopted children were excluded from the sample. Thus, from the original sample, only 233 participants from 76 nuclear families were included in the sample for chapter 4. Of the 233 participants, there were 122 children, 67 mothers, and 44 fathers. Of the 122 children, 83 (68.0%) were male and 32 (26.2%) were female, whilst for 7 children (5.7%) gender was not
indicated. Regarding ADHD diagnosis of children by a healthcare professional as reported by the parents, 88 (72.1%) of the 122 children were diagnosed with ADHD, whilst the remaining 34 (27.9%) had never before been diagnosed with ADHD.

In chapter 5 (Are ADHD combined type and predominantly inattentive type distinct disorders or varying presentations of the same disorder? Perspectives from a family study in a South African sample) siblings from nuclear families within which data was available for at least two family members, were included in the sample. Adopted children were excluded from the sample. Thus, from the original sample, 175 children from 78 nuclear families were included for analysis in chapter 5. Of the 175 children, 110 (62.9%) were male and 65 (37.1%) were female. Regarding ADHD diagnosis by a healthcare professional as reported by the parents, 102 (58.3%) of the 175 children were diagnosed with ADHD, whilst the remaining 73 (41.7%) had never before received a diagnosis of ADHD.

In chapter 6 (Examining the aetiology of the comorbidity of Attention-Deficit/Hyperactivity Disorder and Oppositional Defiant Disorder in a genetically informative sample from South Africa) only children from nuclear families where at least one child was indicated by the parents as having been diagnosed with ADHD by a healthcare professional, were included in the sample. All adopted children were excluded from the sample. Thus, from the original sample, only 164 children from 74 nuclear families were included in the sample for chapter 6. Of the 164 children, 104 (63.4%) were male and 60 (36.6%) were female. Regarding ADHD diagnosis by a healthcare professional as reported by the parents, 101 (61.6%) of the 164 children were diagnosed with ADHD, whilst the remaining 63 (38.4%) children had never before been diagnosed with ADHD.

In chapter 7 (Explaining sex differences in the prevalence of ADHD – testing two models in a South African sample of nuclear families) parents and children from nuclear families were again included in the sample. However, only nuclear families for which data from the mother was available were included. All adopted children were excluded from the sample. Thus, from the original sample, 203 participants from 64 nuclear families were included in the sample for chapter 7, with 101 children, 64 mothers, and 38 fathers. Of the 101 children, 73 (72.3%) were male and 28 (27.7%) were female. Regarding ADHD diagnosis of the children by a healthcare professional as reported by the parents, 74 (73.3%) of the 101 children had
been diagnosed with ADHD, whilst the remaining 27 (26.7%) had never before received a diagnosis of ADHD.

In chapter 8 (Influence of pregnancy and delivery complications on ADHD symptom severity in children) only children from nuclear families where at least one child was indicated by the parents as having been diagnosed with ADHD by a healthcare professional, and for whom data on pregnancy and delivery complications were available, were included in the sample. All singletons and adopted children were excluded from the sample. Thus, from the original sample, only 171 children from 83 nuclear families were included in the analysis for chapter 8. Of the 171 children, 110 (64.3%) were male and 61 (35.7%) were female. Regarding diagnosis of ADHD by a healthcare professional as reported by the parents, 108 (63.2%) of the 171 children were diagnosed with ADHD, whilst the remaining 63 (36.8%) had never before been diagnosed with ADHD.

In chapter 9 (The influence of the interaction between a polymorphism in the dopamine transporter gene and pregnancy and/or delivery complications on the severity of ADHD symptoms in a South African sample) only children for who genotyping results of the DAT1 3’ uVNTR polymorphism, as well as data on pregnancy and delivery complications, were available were included in the sample. Thus, only 51 children were included in the sample, with all adopted children excluded. Of the 51 children, 36 (70.6%) were male and 15 (29.4%) were female. For this subset of the sample, the diagnosing healthcare professional directly confirmed the ADHD diagnostic status of all children reported by the parents as having been diagnosed with ADHD. Of the 51 children, 45 (88.2%) had been diagnosed with ADHD, whilst the remaining 6 (11.8%) children had never before received a diagnosis of ADHD.

In chapter 10 (Testing for the presence of rare sequence variations within the MAOA-uVNTR in a sample of children diagnosed with Attention-Deficit/Hyperactivity Disorder (ADHD)) only children diagnosed with ADHD by a healthcare professional, who had genotype data available for the MAOA-uVNTR polymorphism, and who were not shown to be heterozygous females for this polymorphism, were included in the sample. For this subset of the sample, the diagnosing healthcare professional directly confirmed the ADHD diagnostic status of all the children. Thus, from the original sample, only 33 children were
included in the analysis for chapter 10. Of the 33 children, 29 (87.9%) were male and 4 (12.1%) were female.

Figure 3.1 Flowchart with sample layout
Chapter 4

Familial aggregation of Attention-Deficit/Hyperactivity Disorder (ADHD) subtypes in a South African sample

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This chapter on the familial aggregation of Attention-Deficit/Hyperactivity Disorder (ADHD) in a South African sample was conducted to elucidate whether inattention and hyperactivity runs in families. Since no studies of this nature have been conducted in South Africa, this is a crucial first step which will determine whether family studies can indeed be used, as explained in Chapter 2, to shed some light on the conflicting findings found thus far in molecular genetic studies of ADHD. The research conducted, the statistical analysis, and the writing of this chapter was done by Nadia Fouché.

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Abstract

Attention-Deficit/Hyperactivity Disorder is a highly prevalent childhood developmental disorder. The disorder is characterised by a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning in a number of domains. Although research on the relative contribution of genetic factors, as well as the type of genetic influence, to the aetiology of the disorder is abundant, limited information is available for the South African context. This study aims to fill that gap by making use of a cross-sectional family study design in a South African sample. Familial aggregation of symptoms would be indicative of additive genetic factors influencing a symptom domain, as opposed to non-additive genetic factors. The sample consisted of 233 participants from 76 South African nuclear families. Through the use of multilevel modelling, it was determined that inattention and total Attention-Deficit/Hyperactivity Disorder symptoms significantly aggregated in families, but not hyperactivity symptoms. Although in line with results from other family studies, these findings are in contrast to previous findings from twin and adoption studies. These results either suggest that the shared environment plays a larger role in inattention symptoms than suggested up until now, inflating the familial aggregation of these symptoms, or it can be concluded that additive genetic factors play a greater role in inattention and total disorder symptoms compared to hyperactivity/impulsivity symptoms in a South African sample. This may influence future molecular genetic analysis for the disorder in this country.

**Keywords:** Additive genetic factors, Family study, Hyperactivity/Impulsivity, Inattention, Multilevel modelling, Non-additive genetic factors
4.1 Introduction

In the latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (5th ed.; DSM–5; American Psychiatric Association [APA], 2013), Attention-Deficit/Hyperactivity Disorder (ADHD) is defined as “a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development”. First onset of symptoms occurs during childhood, before the age of 12 years. Children diagnosed with ADHD may display symptoms of only hyperactivity-impulsivity, only inattention, or both hyperactivity-impulsivity and inattention. Consequently, the DSM-5 specifies three distinct presentations of the disorder, namely combined presentation, predominantly inattentive presentation, and predominantly hyperactive-impulsive presentation.

With an estimated world-wide prevalence of approximately 5% (Polanczyk, De Lima, Horta, Biederman, & Rohde, 2007), ADHD is viewed as one of the most widespread and frequently occurring childhood psychiatric disorders (Mill, & Petronis, 2008). The negative ramifications of the disorder are well established, with not only the diagnosed child being affected, but also the child’s family (Harpin, 2005). Children with ADHD tend to struggle academically, achieving lower grades, as well as more frequently repeating grades. In addition, children with ADHD more frequently receive some sort of punishment in school, are expelled more frequently, and show relatively low rates of high school graduation and post-secondary education (Loe, & Feldman, 2007). Apart from negative academic outcomes, children with ADHD also frequently experience problems relating to their peers (Hoza, 2007; Hoza, Mrug, Gerdes, Hinshaw, Bukowski, Gold, et al., 2005; Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012). These problems include peer rejection, and having fewer dyadic friends than children not diagnosed with ADHD (Hoza, Mrug, Gerdes, Hinshaw, Bukowski, Gold, et al., 2005; Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012). Due to the crucial role that interactions with peers play in a child’s development (Hoza, 2007; Rubin, Bukowski, & Parker, 2007), it is not surprising that the peer rejection experienced by the diagnosed child is associated with negative long-term outcomes such as cigarette smoking, delinquency, anxiety, and global impairment (Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012).
Familial aggregation of ADHD subtypes

In addition, research has shown that families of children diagnosed with ADHD experience greater family dysfunction. This normally manifests as severe marital discord, lower levels of cohesiveness and organisation, and more conflict (Foley, 2011; Pheula, Rohde, & Schmitz, 2011). Parenting is also affected, with less parental warmth, less consistent parenting, and a more hostile parenting style being commonly reported (Cussen, Sciberras, Ukoumunne, & Efron, 2012).

The prevalence of the disorder, along with the negative consequences it has on the child and the child’s family, make it a very real public health concern. Although effective pharmaceutical (Greenhill, Swanson, Vitiello, Davies, Clevenger, Wu, et al., 2001; Gunter, 2013) and behavioural (Daley, Van Der Oord, Ferrin, Danckaerts, Doepfner, Cortese, et al., 2014) treatment strategies are available, there is still room for improvement. For instance, stimulants (the first line pharmaceutical treatment for ADHD) have been proven to have an approximate positive response rate of 70% (Greenhill, Swanson, Vitiello, Davies, Clevenger, Wu, et al., 2001; Gunter, 2013). Though impressive, that still means that 30% of children do not benefit significantly from this treatment.

Knowing the aetiology of a disorder is crucial for the development of better treatment strategies. Great strides have been made in this regard with ADHD, especially in discerning the overall effects of genes and the environment respectively. Family, twin, and adoption studies have consistently shown ADHD to be a highly heritable disorder (Biederman, 2005; Faraone, & Doyle, 2001; Lichtenstein, Carlström, Råstam, Gillberg, & Anckarsäter, 2010; Nikolas, & Burt 2010; Sprich, Biederman, Crawford, Mundy, & Faraone, 2000; Takeda, Stotesbery, Power, Ambrosini, Berrettini, Hakonarson, et al., 2010; Thapar, Cooper, Jefferies, & Stergiakouli, 2012). A meta-analysis of twin and adoption studies conducted (Nikolas, & Burt, 2010), found that genetic factors accounted for 71% and 73% of the variance in inattention and hyperactivity scores respectively. It is noteworthy, however, that apart from a recently published study which took into account the effect of maternal ADHD (Van Dyk, Springer, Kidd, Steyn, Solomons, & Toorn, 2014), no family, twin, or adoption studies of this nature have been carried out in the South African population. Moreover, evidence exists that shows significant heterogeneity in the underlying genetic factors causing ADHD in different world populations (Ogdie, Bakker, Fisher, Francks, Yang, Cantor, et al., 2005; Zhou,
Dempfle, Arcos-Burgos, Bakker, Banaschewski, Biederman, et al., 2008). Generalisation of findings between world populations is thus not advised. In order to truly understand the genetic basis of the disorder, conducting independent studies worldwide is crucial (Schuler, Weiss, Chavira, McGough, Berrocal, Sheppard, et al., 2012).

Family studies are frequently the first line of enquiry when looking at possible genetic influences on a trait or disorder. However, since family members usually share an environment as well as genes, similarities between family members cannot be interpreted as being solely due to the influence of genetic factors. The shared environment will also result in similarities (Plomin, & Daniels, 2011). That said, family studies can still provide valuable information regarding the limits of genetic and environmental influences on a trait (Plomin, & Daniels, 2011), and is therefore useful in exploratory type analysis such as the current study. It is important, however, to ensure that results from family studies are not misinterpreted. Only if a trait is influenced by additive genetic effects, or by the shared environment, will there be similarities between first-degree relatives for that trait. Non-additive genetic effects will not necessarily make first-degree relatives more similar to each other. Thus, lack of similarity between relatives for a particular trait could be due to no genetic or shared environmental factors influencing the trait, but could also be the result of only non-additive genetic factors influencing the trait (Nikolas, & Burt, 2010; Plomin, & Daniels, 2011). Although this is a drawback of the family-study design, it also presents an opportunity. Should similarities be found between first-degree relatives, either additive genetic factors, shared environmental factors, or both, are probably at play. Concerning the symptom dimensions of hyperactivity-impulsivity and inattention, the meta-analysis performed on twin and adoption studies indicated that additive genetic factors have a greater influence on hyperactivity-impulsivity than inattention symptom dimensions (Nikolas, & Burt, 2010). Conversely, non-additive genetic influences played a greater role in inattention than hyperactivity-impulsivity. Furthermore, the influence of the shared environment was found to be negligible (Nikolas, & Burt, 2010). These findings are in contrast to findings from studies examining familial clustering of ADHD symptoms. Using quantitative measures of ADHD subtypes, correlations between first-degree relatives (sibling-pairs) were higher for the inattention symptom dimension than for the hyperactive-impulsive symptom dimension (Smalley, McGough, Del’Homme, NewDelman, Gordon, Kim,
et al., 2000). Similarly, in a more recent study, it was found that parental ADHD had a greater effect on inattentive symptoms than hyperactive-impulsive symptoms (Takeda, Stotesbery, Power, Ambrosini, Berrettini, Hakonarson, et al., 2010). This would suggest a greater influence of either the shared environment or additive-genetic factors on inattention than hyperactivity-impulsivity.

The only study taking familial factors into account conducted thus far in South Africa showed that the occurrence of maternal ADHD was significantly higher in children diagnosed with ADHD than in a control group (Van Dyk, Springer, Kidd, Steyn, Solomons, & Toorn, 2014). The latter study, however, only considered global ADHD scores, and did not examine the different subtypes of the disorder. Thus, the aim of the current study will be to look at the familial aggregation of global, hyperactive-impulsive, and inattentive ADHD symptoms in a sample of South African families, taking into account proband, sibling, maternal, and paternal ADHD symptoms. Should additive genetic effects have a greater influence on hyperactivity-impulsivity and the influence of the shared environment be negligible, as found by Nikolas and Burt (2010), latter symptoms will aggregate more between first-degree relatives than inattention symptoms.

4.2 Methods
4.2.1 Participants

The sample consisted of 233 participants from 76 nuclear families, each with at least one child either diagnosed with ADHD, or strongly suspected by the parents of having ADHD. Due to the considerable cost and time implications of formal structured diagnosis by healthcare providers, and as was done in previous studies on ADHD (e.g. LeFever, Villers, & Morrow, 2002; Braun, Kahn, Froehlich, Auinger, & Lanphear, 2006), this study utilised parent-report of child diagnosis by a healthcare provider to classify children as being diagnosed with ADHD or not. Children for whom the parents indicated that they had been diagnosed with ADHD by a healthcare professional were classified as having the disorder, whilst children who the parents indicated had not been diagnosed with ADHD by a healthcare professional were classified as not having ADHD. Only children between the ages of 5 and 18 were included in the sample. Due to the exploratory nature of the study, no further exclusion criteria were applied. There were 122 children, 67 mothers, and 44 fathers. Of the 122 children, 88 were
diagnosed with ADHD, nine were suspected of having ADHD, and the remaining 25 were unaffected siblings. All participants were recruited through social media pages via the Attention Deficit and Hyperactivity Support Group of Southern Africa (ADHASA).

### 4.2.2 Procedures

Parents of children diagnosed with ADHD or suspected of having ADHD were given an information leaflet to read (Appendix A1), with an informed consent form to sign (Appendix A2). Hereafter they were asked to complete questionnaires measuring ADHD symptoms, as well as a number of biographical questions (Appendices B1 and B3), for both themselves and their children. Parents were asked to complete questionnaires for all of their children, regardless of whether they have been diagnosed with ADHD or not. The following measuring instruments were used: 

(a) The SNAP-IV 26-item Teacher and Parent Rating Scale (Swanson, Kraemer, Hinshaw, Arnold, Conners, Abikoff, et al., 2001) (parent version) which measures symptoms of ADHD in children (Appendix B2). The questionnaire is based on the DSM-IV criteria for ADHD in children and consists of items covering both the inattention and hyperactivity/impulsivity symptom domains. Although the DSM-5 criteria for ADHD have been published, there are no essential differences in the symptoms listed in the DSM-IV (APA, 1994) and DSM-5 (APA, 2013), and therefore SNAP-IV was not seen as being an outdated instrument. A total score for ADHD can be derived by combining the two subscale scores. In addition, items measuring Oppositional Defiant Disorder (ODD), a condition frequently comorbid with ADHD, are included in the questionnaire. These latter items were, however, not utilised in the current chapter. The scale consists of 26 items in total, each rated on a 4-point Likert scale, measuring severity of symptoms, and ranging from 0 (Not at all) to 3 (Very much).

(b) The Adult ADHD Self-Report Scale (ASRSv1.1) Symptom Checklist (Kessler, Adler, Ames, Demler, Faraone, Hiripi, et al., 2005) was developed by the World Health Organisation (WHO) with the aim of providing a valid self-assessment measure to screen adults for ADHD (Kessler, Adler, Ames, Demler, Faraone, Hiripi, et al., 2005) (Appendix B4). The scale consists of 18 items, which, similar to the SNAP-IV, were derived from the DSM-IV criteria for ADHD diagnoses. The questions do, however, use adult specific language and is rated by means of a 5-point Likert scale, ranging from 0 (Never) to 4 (Very often).
4.2.3 Statistical analysis

Descriptive statistics were calculated for all demographic variables. In addition, internal consistency reliability coefficients were calculated for all the scales and subscales of the SNAP-IV and ASRS measuring instruments. Familial aggregation of ADHD symptoms was determined for each of the inattentive and hyperactive-impulsive subtypes separately, as well as for the total ADHD scores. In order to ensure that any similarities between family members were not solely due to demographic factors, it was decided to control for age, home language, and gender in the analysis. The degree of familial aggregation was determined through calculating the influence of belonging to a particular family on inattention, hyperactivity-impulsivity, and total ADHD scores, through the construction and comparison of multilevel models. Inattention, hyperactivity-impulsivity, and total ADHD scores were entered as dependent variables in the models built. Parent and child ADHD symptom scores were standardised to ensure comparability between the ASRS and the SNAP-IV scale scores. Since multilevel models account for the effects of missing data (Field, 2013), no data imputation techniques were used to replace missing data in the dataset. Firstly, models were built with either inattention, hyperactivity-impulsivity, or total ADHD symptom scores as dependent variables, and only level one control variables (age, gender, and home language) as predictors. These initial models did not take the influence of family structure into account. All three predictor variables were entered as fixed effects in the model, with age entered as a covariate, and gender and home language entered as factors. Age was grand-mean centred prior to entry into the model as is common practice in multilevel models (Field, 2013). Thereafter, second models that did take family structure into account were built for each of the inattention, hyperactivity-impulsivity, and total ADHD symptom dimensions by adding the level two variable of belonging to a specific family (henceforth called the “Family” variable) as a random effect with varying intercepts to the first models. The two models were then compared for each of the three symptom domains to determine whether the addition of the “Family” variable made a significant improvement in the fit of the models. This was done through the comparison of the -2 Log Likelihood values of the two models by means of a Chi-Square test of statistical significance. A significant Chi-Square test indicated that there was a significant improvement in the fit of the model after the addition of the level two “Family” variable. Intraclass correlation coefficients
were then also calculated from the second models as a ratio of family level variance to total variance in order to determine the percentage of variance explained by belonging to a particular family. Larger intraclass correlation coefficients would be indicative of greater between-family variance, and thus less within-family variance. Consequently, a higher intraclass correlation coefficient would be indicative of a greater degree of familial aggregation of a trait.

The significance level for the tests was set at an alpha value of 0.05, and all tests were two-sided (see Appendix E1 for extracts of the statistical analysis).

4.3 Results
4.3.1 Demographic characteristics

The demographic characteristics of the family members, stratified by gender, are summarised in Table 4.1.

*Table 4.1. Demographic characteristics of the sample. The actual number is followed by the percentage in parenthesis.*

<table>
<thead>
<tr>
<th></th>
<th>Fathers</th>
<th>Mothers</th>
<th>Sons</th>
<th>Daughters</th>
<th>Gender not indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of individuals</td>
<td>44</td>
<td>67</td>
<td>83</td>
<td>32</td>
<td>7</td>
</tr>
<tr>
<td>Age (Mean ± SD)</td>
<td>43 ± 7</td>
<td>38 ± 5</td>
<td>9 ± 3</td>
<td>11 ± 4</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Home language</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afrikaans</td>
<td>18 (40.9%)</td>
<td>24 (35.8%)</td>
<td>26 (31.3%)</td>
<td>11 (34.4%)</td>
<td>3 (42.9%)</td>
</tr>
<tr>
<td>English</td>
<td>19 (43.2%)</td>
<td>35 (52.2%)</td>
<td>38 (45.8%)</td>
<td>14 (43.8%)</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td>Afrikaans and English</td>
<td>6 (13.6%)</td>
<td>6 (9.0%)</td>
<td>7 (8.4%)</td>
<td>2 (6.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Sesotho</td>
<td>0 (0%)</td>
<td>1 (1.5%)</td>
<td>2 (2.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Home language not indicated</td>
<td>1 (2.3%)</td>
<td>1 (1.5%)</td>
<td>10 (12.0%)</td>
<td>5 (15.6%)</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>41 (93.2%)</td>
<td>62 (92.5%)</td>
<td>68 (81.9%)</td>
<td>27 (84.4%)</td>
<td>5 (71.4%)</td>
</tr>
</tbody>
</table>
The majority of participants were white (85.0%), and either Afrikaans or English speaking (88.4%), and thus the sample was not representative of the South African population. The response from fathers was low when compared to that of mothers, with only 44 of the 76 families participating in the study including responses from fathers. In comparison, 67 mothers completed questionnaires for themselves.

4.3.2 Reliability of measuring instruments

The SNAP-IV scale has previously been shown to have adequate internal consistency reliability with Bussing, Fernandez, Harwood, Wei, Garvan, Eyberg, et al. (2008) finding alpha coefficients of 0.90 for inattentive items, 0.79 for hyperactive/impulsive items, and 0.94 for the total scale in a sample of elementary school students in the United States of America. Studies from South Africa using the SNAP-IV measure have been published, but reliability estimates for the subscales and total scale have, subsequent to this study, not yet been reported (Walker, Venter, Van der Walt, & Esterhuyse, 2011; Zeegers, Rabie, Swanevelder, Edson, Cotton, & Van Toorn, 2010). In line with the above results, the total scale and subscales of the SNAP-IV showed very good internal consistency reliability in the current study. Cronbach’s alpha coefficients greater than 0.9 for each of the subscales and total scale were found (Inattention: α=0.957; Hyperactivity/impulsivity: α=0.933; Total ADHD: α=0.962).

Adequate internal consistency reliability coefficients have also been found in previous studies for the ASRS, with alpha coefficients ranging from 0.93 to 0.94 (Adler, Shaw, Spencer, Newcorn, Hammerness, Sitt, et al., 2012). In addition, a previous study in South Africa found the ASRS to be a reliable measure of adult ADHD, with an alpha coefficient of 0.886 reported in a student population (Burke, Austin, & Waldeck, 2011). Likewise, in the current study, all the subscales and the total scale of the ASRS had Cronbach’s alpha values greater than 0.9 (Inattention: α=0.931; Hyperactivity/impulsivity: α=0.916; Total ADHD: α=0.951). The SNAP-
IV and ASRS can consequently be viewed as reliable measures of ADHD symptom dimensions in children and adults respectively.

### 4.3.3 Comparison of the multilevel models

The variables entered in each of the multilevel models that were compared to determine whether familial aggregation was present for each of the inattention, hyperactivity/impulsivity, and total ADHD symptom domains, are summarised in Table 4.2.

The results for the comparison of the two multilevel models are presented below for each of the inattention, hyperactivity/impulsivity, and total ADHD symptom domains (Table 4.3). As described under statistical techniques, for model one, only the control variables (age, home language, and gender) were entered without taking family structure into account. Model two consisted of the same variables as model one, but this time family structure was taken into account.

*Table 4.2. Variables entered in the multilevel models to be compared.*

<table>
<thead>
<tr>
<th></th>
<th>Total ADHD</th>
<th>Hyperactivity/Impulsivity</th>
<th>Inattention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fixed effects</strong></td>
<td>Gender</td>
<td>Gender</td>
<td>Gender</td>
</tr>
<tr>
<td>Home language</td>
<td>Home language</td>
<td>Home language</td>
<td>Home language</td>
</tr>
<tr>
<td>Age</td>
<td>Age</td>
<td>Age</td>
<td>Age</td>
</tr>
<tr>
<td><strong>Random effects</strong></td>
<td>None</td>
<td>Family</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Family</td>
</tr>
</tbody>
</table>
Table 4.3. Results of model comparison for ADHD symptom domains, adjusted for age, home language, and gender.

<table>
<thead>
<tr>
<th>Symptom Domain</th>
<th>Minus 2 Log Likelihood value for Model 1</th>
<th>Minus 2 Log Likelihood value for Model 2</th>
<th>Chi-square change</th>
<th>Degrees of freedom</th>
<th>Intraclass correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ADHD</td>
<td>578.40</td>
<td>573.20</td>
<td>5.20*</td>
<td>1</td>
<td>0.15</td>
</tr>
<tr>
<td>Hyperactivity/impulsivity</td>
<td>575.12</td>
<td>573.15</td>
<td>1.98</td>
<td>1</td>
<td>0.09</td>
</tr>
<tr>
<td>Inattention</td>
<td>576.18</td>
<td>570.33</td>
<td>5.85*</td>
<td>1</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*p<0.05 (Critical value for Chi-square statistic at 1 degree of freedom = 3.84)

The addition of family structure resulted in a significant improvement in model fit for the total ADHD and inattention symptom domains (p<0.05), but not for the hyperactivity symptom domain (p>0.05) (Table 4.3). Intraclass correlation coefficients of 0.15 and 0.17 were found for Total ADHD and Inattention scores respectively. Hyperactivity/impulsivity showed an intraclass correlation coefficient of only 0.09.

4.4 Discussion

Cronbach alpha values for the scales were comparative to that of previous studies, and thus show that the SNAP-IV and ASRS are reliable for use in South Africa. The descriptive statistics clearly showed that the sample was not representative of the South African population, and thus results should not be generalised to the population at large. This is common in published studies in behavioural genetic research (Agudelo, Gálvez, Fonseca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Van Dyk, Springer, Kidd, Steyn, Solomons, & Van Toorn, 2014), but nonetheless prevents generalization of results to the South African population at large.

The results from the current study contradict the findings from the meta-analysis conducted by Nikolas and Burt (2010), where additive genetic factors were found to have a greater influence on hyperactivity/impulsivity than inattention symptoms, and the influence of the shared environment was found to be negligible. In the current study, comparison of the multilevel models showed greater similarities between family members for inattention
and total ADHD symptoms as compared to hyperactivity/impulsivity symptoms. As previously mentioned, similarities between first-degree relatives are indicative of additive genetic factors, shared environmental factors, or both, influencing a trait (Plomin, & Daniels, 2011). Multilevel modelling showed that differences between members from different families accounted for a significant percentage of the variance in the total ADHD and inattention symptom domains, but not for the hyperactive/impulsive symptom domain. The intraclass correlation coefficients provided a measure of the size of these effects. For total ADHD and inattention scores, 15% and 17% of the variance respectively can be attributed to differences between members from different families. For hyperactivity/impulsivity, only 9% of the variance could be attributed to the latter named differences. It thus appears that there is a greater degree of familial aggregation, and consequently a greater influence of additive and/or shared environmental factors, for the inattention and total ADHD symptom domains when compared to the hyperactivity symptom domain.

It is important to keep in mind that total ADHD is a composite of scores of the inattention and hyperactivity symptom domains. Consequently, it appears that the significant familial aggregation observed for total ADHD is mostly due to the familial aggregation of inattention symptoms as opposed to hyperactivity/impulsivity symptoms. Recall that just because additive genetic variance does not significantly influence a trait, it does not mean that genetics do not play a role in that trait. Non-additive genetic factors may still be at play, since these will not necessarily lead to similarities between family members (Nikolas, & Burt, 2010; Plomin, & Daniels, 2011).

Although in contrast to the findings from Nikolas and Burt (2010), the above findings are in line with that from Smalley, McGough, Del’Homme, NewDelman, Gordon, Kim, et al. (2000) and Takeda, Stotesbery, Power, Ambrosini, Berrettini, Hakonarson, et al. (2010) who also found the greatest degree of similarity between first-degree relatives for the inattention symptom domain. Though comparing only siblings instead of full nuclear families, these researchers made use of the same rating scale (SNAP-IV) that was used in the current study to measure childhood ADHD. Unlike the Nikolas and Burt (2010) meta-analysis based on twin and adoption studies, familial aggregation studies like the current one and that conducted by Smalley, McGough, Del’Homme, NewDelman, Gordon, Kim, et al. (2000) and Takeda,
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Stotesbery, Power, Ambrosini, Berrettini, Hakonarson, et al. (2010) cannot separate the influence of additive genetic factors and the shared environment. It is possible that the conflicting findings are due, not to greater additive genetic factors influencing inattention compared to hyperactivity/impulsivity, but rather a greater influence of the shared environment on inattention symptoms. Consequently, it may be argued that inattention is indeed influenced to a lesser extent by additive genetic factors than hyperactivity/impulsivity, as found in twin and adoption studies. The greater influence of the shared environment would lead to an inflation of the similarities between first-degree relatives, resulting in greater familial aggregation for inattention than hyperactivity/impulsivity. That said, as previously noted, studies have thus far found the effects of shared environmental factors on ADHD symptom dimensions to be negligible (Burt, 2009; Nikolas, & Burt 2010). However, the minimal influence of the shared environment has recently been questioned (Wood, Buitelaar, Rijsdijk, Asherson, & Kuntsi, 2010), and it is possible that it may have a greater influence than originally thought.

The results from this study may also be influenced by the limitations of the study, of which a primary one is the predominance of families where mothers, but not fathers, responded. Takeda, Stotesbery, Power, Ambrosini, Berrettini, Hakonarson, et al. (2010) deduced from their results that hyperactivity/impulsivity seems to be influenced to a greater extent by paternal ADHD symptoms, rather than maternal ADHD. The limited number of fathers in the sample may therefore result in an underestimation of the degree of familial aggregation of the hyperactivity/impulsivity symptom domain.

4.5 Conclusion

In this study, inattention symptoms and total ADHD symptom scores, but not hyperactivity/impulsivity symptoms, were found to significantly aggregate in families with at least one child either diagnosed with ADHD, or strongly suspected of having ADHD. Although in contrast to findings from twin and adoption studies, these results are in line with findings from studies of familial aggregation of ADHD symptom dimensions. This pattern of results may indicate that the shared environment plays a greater role in inattention symptoms than previously thought. Should this not be the case, it may also indicate that additive genetic factors do indeed play a bigger role in influencing inattention symptoms compared to
hyperactive/impulsive symptoms in this South African sample. This could inform future molecular genetic studies of the disorder in this country by focusing on the epistatic effects of genes at different loci when examining hyperactivity/impulsivity, whilst looking at additive genetic influences when examining inattention.

4.6 Limitations of the study

Since it was the first study of its kind to be conducted in a South African sample, the study was primarily exploratory in nature. Therefore, exclusion criteria were relaxed, and all children between the ages of 5 and 18 who were either diagnosed with ADHD or strongly suspected of having ADHD, and whose families were willing to participate, were included in the sample. Future studies may need to look at enforcing more stringent exclusion criteria. In addition, as previously mentioned, there was an overrepresentation of families with only the symptom scores of mothers, but not fathers in the sample. Care should be taken in future to ensure that more fathers respond in order to examine the differential effects of maternal and paternal ADHD symptomology on the symptoms of their children.

A further limitation to this study is that the sample was not representative of the South African population, due to the non-probability sampling process through social media pages that was employed. The findings from this study can therefore not be generalised to the broader South African population, but the statistical and family-study methods employed should rather serve to inform future research in this country utilising representative samples. A final limitation to this study is that classification of children as diagnosed with ADHD or not were made according to parent-report of diagnosis by a healthcare provider. Although structured diagnostic interviews directly performed by a healthcare provider is preferable for diagnostic classification of participants, the time and costs involved in this process fell outside the time allocated, or budget available, for the study. However, lending some credence to the methodology employed here is a study by Visser, Danielson, Bitsko, Perou, and Blumberg (2013) in which it was found that prevalence rates of ADHD as deduced from parent-report of healthcare provider diagnosis was statistically indistinguishable from that of documented ADHD diagnosis in medical records of healthcare providers.
Chapter 5

Are ADHD combined type and predominantly inattentive type distinct disorders or varying presentations of the same disorder? Perspectives from a family study in a South African sample

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This chapter is the first in the thesis to explore a possible reason for the mixed findings in the molecular genetic studies on Attention-Deficit/Hyperactivity Disorder (ADHD). Should ADHD combined type and predominantly inattentive type be distinct disorders, rather than subtypes of the same disorder, the aetiology may differ between them. This could result in mixed findings if researchers do not distinguish between the two types in their samples. The research conducted, the statistical analysis, and the writing of this chapter was done by Nadia Fouché.

Acknowledgements: Financial support by the National Research Foundation, South Africa, is greatly acknowledged.
Abstract

There has been a long-standing debate in the literature on whether the Attention-Deficit/Hyperactivity Disorder (ADHD) combined and inattentive subtypes are variations of the same underlying disorder, or distinct disorders. The prevalence and negative consequences of the disorder make research aimed at understanding the distinction between the recognised subtypes both relevant and important for the design of future studies. Insight into this question have been gained from family studies aiming to determine whether the subtypes “breed true” in relatives of diagnosed individuals. Although results from the family study literature point to the subtypes being varying presentations of the same disorder, rather than distinct disorders, no studies of this kind have been conducted in a sample from South Africa thus far. Thus, this study aimed to fill that gap by using a cross-sectional family study design in a South African community sample of nuclear families, enriched with diagnoses of ADHD. Children from 78 nuclear families, each consisting of two or three siblings, were classified into ADHD subgroups (predominantly inattentive type, combined type, and no disorder) based on scores from the DSM-IV derived SNAP-IV rating scale. Recurrence risk in sibling pairs were determined by means of chi-square goodness-of-fit tests comparing the proportion of subtype concordant and discordant pairs. For both the predominantly inattentive and combined subtypes, the proportion of concordant pairs were not significantly greater than the proportion of discordant pairs. This finding from a South African sample concurs with studies from other countries that the inattentive and combined subtypes are varying presentations of the same disorder, rather than distinct disorders. This finding may direct the design of future research into the aetiological nature of the ADHD subtypes in South Africa.

Keywords ADHD combined subtype, ADHD inattentive subtype, Chi-square goodness-of-fit test, Disease concordance and discordance, Family study, Sibling pairs
5.1 Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as “a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development” (5th ed.; DSM–5; American Psychiatric Association [APA], 2013). Onset of symptoms should occur before the age of 12 years. In addition, the disorder has a world-wide prevalence of approximately 5% (Polanczyk, De Lima, Horta, Biederman, & Rohde, 2007), making it one of the most frequently occurring childhood onset psychiatric disorders (Mill, & Petronis, 2008).

The negative ramifications of the disorder are well documented. Academic and other school difficulties (Loe, & Feldman, 2007), problems in peer relations (Hoza, 2007; Hoza, Mrug, Gerdes, Hinshaw, Bukowski, Gold, et al., 2005; Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, etal., 2012), and family dysfunction (Foley, 2011; Pheula, Rohde, & Schmitz, 2011) have all been associated with ADHD. The wide-spread prevalence of the disorder, along with the negative consequences associated with it, makes research aimed at understanding the aetiology of ADHD both relevant and important. Understanding the aetiology, however, is made complicated by the heterogeneous nature of the disorder. Children diagnosed with ADHD may display symptoms of only hyperactivity-impulsivity, only inattention, or both hyperactivity-impulsivity and inattention. Consequently, three distinct presentations of the disorder (also referred to as subtypes) have been identified, namely combined presentation, predominantly inattentive presentation, and predominantly hyperactive-impulsive presentation (APA, 2013). These differing presentations of the disorder will henceforth be referred to as the inattentive, hyperactive-impulsive, and combined subtypes of ADHD. In a meta-analysis, Willcutt (2012) reported the inattentive subtype to be the most prevalent subtype when subtype classification is based on parent reporting, followed by the combined subtype, and finally the hyperactive-impulsive subtype. However, the researcher found that individuals with the combined subtype are the most likely to be referred for clinical services.

A great point of contention in the literature is whether these subtypes truly represent varying presentations of the same disorder, or are in fact distinct disorders (Diamond, 2005; Faraone, Biederman, & Friedman, 2000; Faraone, Biederman, Mick, Williamson, Wilens, Spencer, et al., 2000; Milich, Balentine, & Lynam, 2001; Nigg, Tannock, & Rohde, 2010;
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Smalley, McGough, Del'Homme, NewDelman, Gordon, Kim, et al., 2000; Stawicki, Nigg, & Von Eye, 2006; Todd, Rasmussen, Neuman, Reich, Hudziak, Bucholz, et al., 2001; Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al., 2012; Woo, & Rey, 2005). Due to the low prevalence of the hyperactive-impulsive subtype, particular attention has been paid to the distinction between the inattentive and combined subtypes of ADHD (Diamond, 2005; Faraone, Biederman, & Friedman, 2000; Milich, Balentine, & Lynam, 2001; Stawicki, Nigg, & Von Eye, 2006). Family studies present an opportunity to gain some insight into this question. Should the subtypes simply be variants of the same condition, the subtypes would not be expected to “breed true” in families. That is, children with ADHD combined type would be expected to have relatives who are at equal risk for both ADHD combined type and predominantly inattentive type, and vice versa. This is often referred to as the ‘gradient-of-severity’ hypothesis. In contrast, should the subtypes represent distinct disorders, driven by different aetiological processes, relatives of children with ADHD combined type would also tend to have ADHD combined type, rather than ADHD inattentive type, and vice versa (Faraone, Biederman, & Friedman, 2000; Stawicki, Nigg, & Von Eye, 2006).

Results from previous family studies testing the distinction between the subtypes have been conflicting, although the weight of the evidence points to ADHD combined type and ADHD inattentive type simply being variations of the same disorder. Studies classifying subtypes according to criteria stipulated in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (4th ed.; DSM–IV; American Psychiatric Association [APA], 1994) found that the ADHD combined and inattentive subtypes do not breed true, with no association between the subtype of the affected child and that of his/her relatives (Faraone, Biederman, Mick, Williamson, Wilens, Spencer, et al., 2000; Faraone, Biederman, & Friedman, 2000; Smalley, McGough, Del'Homme, NewDelman, Gordon, Kim, et al., 2000). Two subsequent meta-analysis pooling data from multiple family studies for the most part confirmed these findings. In the first meta-analysis, Stawicki, Nigg, and Von Eye (2006), concluded that some degree of both subtype-specific transmission and non-specific (“gradient”) transmission do occur, although the effect size for non-specific transmission was small. These researchers proposed that ADHD combined type and ADHD inattentive type are partially overlapping conditions, but that they also have some distinct aetiological factors. In a subsequent meta-analysis, Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al.
ADHD combined and predominantly inattentive types

(2012) found that there was insufficient evidence to classify DSM-IV ADHD subgroups as distinct disorders, particularly citing the poor long-term stability that has been found for the subtypes.

In more recent research, the focus has shifted away from family studies to other sources of evidence for determining if ADHD combined type and ADHD inattentive type are distinct disorders. These include studies on the neurostructural, neurofunctional, neurocognitive, molecular genetics, and treatment response similarities/differences between ADHD combined type and ADHD inattentive type. In the neuro-related studies conducted, researchers have shown different white matter microstructural brain abnormalities (Lei, Ma, Du, Shen, Jin, & Gong, 2014), different patterns of atypical neural connectivity (Fair, Nigg, Lyer, Bathula, Mills, Dosenbach, et al., 2013), different task-related neurophysiological impairments (Mazaheri, Fassbender, Coffey-Corina, Hartanto, Schweitzer, & Mangun, 2014), and different neurocognitive deficits (Dovis, Van der Oord, Wiers, & Prins, 2015) between the two subtypes. That said, in all these studies, the researchers also found overlapping neural features between the two subtypes, supporting the conclusion that the subtypes represent variations of the same disorder.

Although few studies have thus far been conducted on pharmacological treatment response differences between the subtypes, a recent study by Beery, Quay, and Pelham (2017) found that methylphenidate (MPH) was more beneficial in the reduction of symptoms for ADHD combined type compared to ADHD inattentive type, and in some cases even had a detrimental effect on problem behaviours in children with predominantly inattentive symptoms. This finding replicates a previous finding by Grizenko, Paci, and Joober (2010), who found a higher frequency of a beneficial MPH response in a group of children with either ADHD combined type or hyperactivity only, compared to an ADHD inattentive type group. However, in a comprehensive meta-analysis, Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al. (2012) concluded that there is little evidence for differential efficacy of treatment between the ADHD combined and inattentive subtypes.

In the same meta-analysis, Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al. (2012) reported that no studies included in the meta-analysis found a significant difference in the association of any candidate genes with ADHD combined type and ADHD inattentive
type, when the two subtypes were compared directly. Surprisingly, there are very few recent studies comparing molecular genetic factors between the ADHD subtypes, with the vast majority of molecular genetic studies not parsing samples into ADHD combined and inattentive subtypes (Gatt, Burton, Williams, & Schofield, 2015; Hawi, Cummins, Tong, Johnson, Lau, Samarrai, et al., 2015; Middeldorp, Hammerschlag, Ouwens, Groen-Blokhuys, St. Pourcain, Greven, et al., 2016). The one study that could be found compared genotype distributions between single nucleotide polymorphisms (SNPs) in the alpha-2A adrenergic receptor gene (ADRA2A) and the catechol-o-methyltransferase gene (COMT) between ADHD inattentive and combined subtypes, but no significant differences were found (Unal, Unal, Alikasifoglu, & Cetinkaya, 2016).

Given the above discussion, it seems that the preponderance of evidence points towards ADHD combined type and ADHD inattentive type being subtypes of the same disorder, rather than distinct disorders. However, to date, no studies have been conducted in a sample from South Africa on whether the subtypes represent distinct disorders, or simply varying presentations of the same disorder. Thus, the aim of the current study was to begin to fill that gap by using statistical methods to investigate the aetiological nature of the two identified subtypes in a South African sample of sibling pairs. In line with the evidence in the aforementioned discussion, it was hypothesised that ADHD combined type and ADHD inattentive type would not “breed true”, and thus represent varying presentations of the same disorder, rather than distinct disorders.

5.2 Methods
5.2.1 Participants

The sample consisted of a South African community sample of 175 children from 78 nuclear families, enriched with diagnoses of ADHD. Diagnosis of ADHD was gleaned by asking parents whether a child has been diagnosed with ADHD by a healthcare professional or not. This methodology has been employed in previous published studies on ADHD (e.g. LeFever, Villers, & Morrow, 2002; Braun, Kahn, Froehlich, Auinger, & Lanphear, 2006), and was decided upon due to the considerable time and cost implications associated with formal structured diagnostic interviews done directly by healthcare providers. Data were available for three siblings from each of 19 of the nuclear families, and for two siblings from each of
the remaining 59 nuclear families. This resulted in a total of 116 sibling pairs that could be compared. Only children between the ages of 5 and 18 years were included in the sample, whilst all adopted children were excluded from the sample. Due to the exploratory nature of the study, no further exclusion criteria were applied.

5.2.2 Procedure

5.2.2.1 Sample recruitment and measuring instruments

Nuclear families were recruited through social media pages via the Attention Deficit and Hyperactivity Support Group of Southern Africa (ADHASA), as well as through medical professionals working with children diagnosed with ADHD in the Bloemfontein area, Free State province, South Africa. Parents were provided with an information leaflet to read (Appendix A1) and an informed consent form to sign (Appendix A2), and, after provision of consent to participate in the study, were asked to complete the following measuring instruments:

(a) The SNAP-IV 26-item Teacher and Parent Rating Scale (Swanson, Kraemer, Hinshaw, Arnold, Conners, Abikoff, et al., 2001) (parent version) is based on the DSM-IV criteria for ADHD in children, covering both symptoms of inattention and hyperactivity/impulsivity (Appendix B2). There are no essential differences in the symptoms listed for ADHD in the DSM-IV (APA, 1994) and the recently published DSM-5 (APA, 2013), and therefore the SNAP-IV was seen as an appropriate instrument for current use. The scale consists of nine items measuring symptoms of inattention and nine items measuring symptoms of hyperactivity/impulsivity. Each of these subsets are summed to obtain total scores for the inattention and hyperactivity/impulsivity symptom domains respectively. A total score for ADHD symptom severity is derived from summing all 18 items. The scale also includes eight items for measuring symptoms of oppositional defiant disorder (ODD), resulting in a total of 26 items. In this chapter, only the 18 items measuring symptoms of ADHD were used for analysis. All items are measured on a four-point Likert scale, ranging from 0 (Not at all) to 3 (Very much).
(b) A self-compiled questionnaire gathering biographical information such as gender and age, as well as a question related to whether the child has been formally diagnosed with ADHD (Appendix B1).

5.2.2.2 Rationale for analysis

As explained in the introduction above, if ADHD combined type and ADHD inattentive type are just variations of the same condition, relatives of participants with ADHD combined type would be just as likely to be classified as ADHD inattentive type as they would be to be classified as ADHD combined type, and vice versa. However, should ADHD combined type and ADHD inattentive type be distinct disorders, relatives of participants with ADHD combined type should be more likely to be classified in the ADHD combined type category than in the ADHD inattentive type category, and vice versa (Faraone, Biederman, & Friedman, 2000; Stawicki, Nigg, & Von Eye, 2006).

Therefore, if ADHD combined type and ADHD inattentive type are variations of the same underlying disorder, in a sample of sibling pairs where at least one member of each pair is classified as ADHD combined type, the proportion of pairs concordant for ADHD combined type (i.e. the proportion of pairs where both members are classified as ADHD combined type) should not differ significantly from the proportion of ADHD combined type / ADHD inattentive type discordant pairs (i.e. the proportion of pairs where one member of the pair is classified as ADHD combined type whilst the other member is classified as ADHD inattentive type). Similarly, in a sample of sibling pairs where at least one member of each pair is classified as ADHD inattentive type, the proportion of pairs concordant for ADHD inattentive type should not differ significantly from the proportion of ADHD combined type / ADHD inattentive type discordant pairs. In contrast, should ADHD combined type and ADHD inattentive type represent distinct disorders, the proportion of sibling pairs concordant for ADHD inattentive type or for ADHD combined type should be greater than the proportion of pairs discordant for ADHD inattentive type / ADHD combined type.

5.2.3 Statistical analysis

Data were cleaned prior to analysis and descriptive statistics calculated for all demographic and categorical variables. Mean imputation was used to replace missing values
for both the hyperactivity/impulsivity and inattention subscales of the SNAP-IV scale (Siddiqui, 2015). Participants had to have answered at least seven out of the nine questions (83% of the nine questions -rounded) for both the inattention and hyperactivity/impulsivity symptom domains for means to be imputed. Any participant who answered fewer than seven out of nine questions for any one of the subscales was excluded from the analysis. To assure the reliability of the measuring instruments, internal consistency reliability was calculated for both the inattention and hyperactivity/impulsivity subscales of the SNAP-IV scale. Data analysis was conducted through the use of the statistical software package, SPSS version 23, and Microsoft Excel 2016.

ADHD subtype classification of participants was done through making use of cut-off scores for the SNAP-IV scale proposed by Swanson (n.d.). Scores on the inattention subdomain were firstly totalled and secondly categorised as “clinically significant inattention” if participants obtained a score of 13 or greater. Similarly, scores on the hyperactivity/impulsivity subdomain were totalled and categorised as “clinically significant hyperactivity/impulsivity” if participants obtained a score of 13 or greater. Participants who obtained scores of less than 13 on the inattention and/or hyperactivity/impulsivity subdomains were categorised as having “inattention symptoms not clinically significant” and/or “hyperactivity/impulsivity symptoms not clinically significant”, respectively. Finally, these newly recoded subdomain scores were combined for each child as follows: “clinically significant inattention” plus “clinically significant hyperactivity/impulsivity” = ADHD combined type; “clinically significant inattention” plus “hyperactivity/impulsivity symptoms not clinically significant” = ADHD inattentive type; “clinically significant hyperactivity/impulsivity” plus “inattention symptoms not clinically significant” = ADHD hyperactive-impulsive type; and “inattention symptoms not clinically significant” and “hyperactivity/impulsivity symptoms not clinically significant” = No disorder. As expected, and in line with previous research (Fair, Nigg, Lyer, Bathula, Mills, Dosenbach, et al., 2013; Skogli, Egeland, Andersen, Hovik, & Øie, 2014; Willcutt, 2012), the number of participants classified as ADHD hyperactive-impulsive type was very low (only three children), and thus these participants were omitted from all further analysis.
The classification of participants as ADHD combined type, ADHD inattentive type, or No disorder were subsequently compared within sibling pairs to determine whether the subtypes “breed true”, as explained in the rationale for analysis section above and in the introduction. This was carried out through firstly identifying the total number of pairs where at least one member of the pair was diagnosed with ADHD combined type, and the total number of pairs where at least one member of the pair was diagnosed with ADHD inattentive type. Secondly, within the group of sibling pairs where at least one child was classified as ADHD combined type, a chi-square goodness-of-fit test was conducted to test whether the concordance rate for ADHD combined type in pairs of siblings was equal to the ADHD combined type plus ADHD inattentive type discordance rate for pairs. The analysis was repeated for the group of sibling pairs where at least one child was classified as ADHD inattentive type, to compare whether the concordance rate for ADHD inattentive type in pairs of siblings was equal to the ADHD combined type plus ADHD inattentive type discordance rate for pairs.

The significance level for the tests was set at an alpha value of 0.05, and all tests were two-sided (see Appendix E2 for extracts of the statistical analysis).

5.3 Results

The average age of the children in the sample was 10 years with 110 (62.9%) males and 65 (37.1%) females (Table 5.1).

*Table 5.1. Demographic characteristics.*

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Mean ± SD)</strong></td>
<td>10 ± 3</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male (Count(%))</td>
<td>110 (62.9%)</td>
</tr>
<tr>
<td>Female (Count(%))</td>
<td>65 (37.1%)</td>
</tr>
</tbody>
</table>
5.3.1 Reliability of measuring instruments

The SNAP-IV scale showed good internal consistency reliability for both the inattention and hyperactivity/impulsivity subscales, with Cronbach’s alpha values of 0.965 and 0.940 for the inattention and hyperactivity/impulsivity subscales respectively.

5.3.2 Diagnostic status and subtype classification of participants

Of the 175 children in the sample, 102 (58.3%) were diagnosed with ADHD by a medical professional, and the remaining 73 (41.7%) were not diagnosed with the disorder, and were thus seen as not having ADHD.

As explained under the statistical analysis section, the subtype classification was based on total scores from the SNAP-IV inattention and hyperactivity/impulsivity subscales (Table 5.2).

Table 5.2. Subtype classification of sample.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No disorder</td>
<td>68</td>
<td>38.9</td>
</tr>
<tr>
<td>ADHD hyperactive-impulsive type</td>
<td>3</td>
<td>1.7</td>
</tr>
<tr>
<td>ADHD inattentive type</td>
<td>31</td>
<td>17.7</td>
</tr>
<tr>
<td>ADHD combined type</td>
<td>73</td>
<td>41.7</td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>100.0</td>
</tr>
</tbody>
</table>

ADHD combined type was the most frequently occurring subtype classification in this sample, with 73 (41.7%) children classified in this category. A further 31 children (17.7%) were classified under the ADHD inattentive type category, whilst only three (1.7%) were classified under the ADHD hyperactive-impulsive type category. The remaining 38.9% of the sample did not meet the criteria for any of the categories, and were thus classified as having no disorder (Table 5.2). Due to the low number of participants classified as ADHD hyperactive-impulsive type, this category was not taken into account in any further analysis.
Since one of the participants classified as ADHD hyperactive-impulsive type came from a family for which data were available for three siblings, whilst the other two falling in this category came from two-sibling families, the exclusion of the ADHD hyperactive-impulsive type category resulted in the exclusion of four sibling pairs from all subsequent analysis.

5.3.3 Analysis results for ADHD combined type and ADHD inattentive type as distinct disorders / variations of the same disorder

5.3.3.1 ADHD combined subtype

Out of a total of 112 sibling pairs, 76 pairs had at least one member who was classified under the ADHD combined type category. Of the 76 pairs, 17 (22%) were concordant for ADHD combined type, with both siblings in these pairs classified under the ADHD combined type category. The remaining 59 pairs were discordant for the subtype categories, with 45 pairs (59%) comprised of one ADHD combined type sibling and one sibling with no disorder, and 14 pairs (18%) comprised of one ADHD combined type sibling and one ADHD inattentive type sibling.

The proportion of pairs concordant for ADHD combined type was compared to the proportion of pairs discordant for ADHD combined type and ADHD inattentive type by means of a chi-square goodness-of-fit test (Table 5.3). The values in the Expected N column in Table 5.3 is an indication of how many pairs of siblings would need to fall into each of the two categories (concordant versus discordant pairs) for there to be no difference in the proportions of concordant and discordant sibling pairs. Thus, for the 31 pairs of siblings who were either concordant for ADHD combined type or discordant for ADHD inattentive and ADHD combined type, 15.5 pairs would need to fall into each category for the proportions to be equal (Table 5.3). For the chi-square goodness-of-fit test to be statistically significant (i.e. indicate that the proportions are not equal), the actual observed frequencies of pairs in the concordant and discordant categories should deviate substantially from the expected frequencies. The observed frequencies were 17 (54.8%) sibling pairs concordant for ADHD combined type and 14 (45.2%) sibling pairs discordant (one member ADHD combined type and one member ADHD inattentive type) (Table 5.3).
Table 5.3. Observed versus expected frequencies for ADHD combined type concordant and ADHD combined/ADHD inattentive type discordant sibling pairs.

<table>
<thead>
<tr>
<th></th>
<th>Observed N</th>
<th>Expected N</th>
<th>Residual</th>
<th>Chi-square goodness-of-fit test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chi-square value</td>
</tr>
<tr>
<td>Concordant pairs (both members ADHD combined type)</td>
<td>17 (54.8%)</td>
<td>15.5 (50.0%)</td>
<td>1.5</td>
<td>0.290</td>
</tr>
<tr>
<td>Discordant pairs (one member ADHD combined type, one member ADHD inattentive type)</td>
<td>14 (45.2%)</td>
<td>15.5 (50.0%)</td>
<td>-1.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31 (100.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results of the chi-square goodness-of-fit test shows that there were no statistically significant differences in the observed versus the expected frequencies [$\chi^2(1) = 0.290, p = 0.590$] (Table 5.3). Therefore, it can be concluded that the proportion of ADHD combined type concordant pairs was not significantly different from the proportion of ADHD combined type / ADHD inattentive type discordant pairs in this group of 31 sibling pairs.

5.3.3.2 ADHD predominantly inattentive subtype

Out of a total of 112 sibling pairs, 37 pairs had at least one member who were classified under the ADHD inattentive type category. Of the 37 pairs, only one pair (3%) was concordant for ADHD inattentive type, with both siblings in this pair classified in the ADHD predominantly inattentive type category. The remaining 36 pairs were discordant for the subtype categories, with 22 pairs (59%) comprised of one ADHD inattentive type sibling and one sibling with no disorder, and 14 pairs (38%) comprised of one ADHD inattentive type sibling and one ADHD combined type sibling.

The proportion of pairs concordant for ADHD inattentive type were compared to the proportion of pairs discordant for ADHD inattentive type and ADHD combined type by means of a chi-square goodness-of-fit test. The chi-square goodness-of-fit test shows that there
were significant differences in the observed versus the expected frequencies \( \chi^2(1) = 11.267, p = 0.001 \) (Table 5.4). Therefore, the proportion of sibling pairs concordant for ADHD inattentive type was not equal to the proportion of discordant ADHD inattentive type/ADHD combined type sibling pairs. However, contrary to what would be expected if ADHD inattentive type and ADHD combined type represented distinct disorders, the proportion of ADHD inattentive type concordant pairs were far smaller (6.7%) than the proportion of ADHD inattentive type/ADHD combined type discordant sibling pairs (93.3%) (Table 5.4).

Table 5.4. Observed versus expected frequencies for ADHD inattentive type concordant and ADHD inattentive type/ADHD combined type discordant sibling pairs.

<table>
<thead>
<tr>
<th></th>
<th>Observed N</th>
<th>Expected N</th>
<th>Residual</th>
<th>Chi-square goodness-of-fit test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chi-square value</td>
</tr>
<tr>
<td>Concordant pairs (both members ADHD inattentive type)</td>
<td>1 (6.7%)</td>
<td>7.5 (50.0%)</td>
<td>-6.5</td>
<td>11.267</td>
</tr>
<tr>
<td>Discordant pairs (one member ADHD inattentive type, one member ADHD combined type)</td>
<td>14 (93.3%)</td>
<td>7.5 (50.0%)</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15 (100.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.4 Discussion

This study aimed to test whether the subtypes of ADHD identified in the DSM-5 as combined presentation and predominantly inattentive presentation are truly variations of the same disorder, or rather distinct disorders, by means of a family study design. In accordance with proposals by researchers in earlier studies (Faraone, Biederman, & Friedman, 2000; Stawicki, Nigg, & Von Eye, 2006), we hypothesised that equal rates of concordance and discordance for a subtype in a sample of sibling pairs would be indicative
of the subtypes not “breeding true” in relatives of affected individuals, and therefore point to ADHD inattentive type and ADHD combined type simply being variations of the same disorder. In contrast, should there be a significantly greater rate of subtype concordance than discordance in said sample, it would indicate that ADHD inattentive type and ADHD combined type “breed true” in families, and thus represent distinct disorders.

In contrast to the findings from the meta-analysis conducted by Willcutt (2012) where ADHD inattentive type was found to be the most frequently occurring subtype, ADHD combined type was the most frequently classified subtype in the current study, followed by ADHD inattentive type, and then ADHD hyperactive-impulsive type. Willcut (2012) did however find that individuals with ADHD combined type are more likely to be referred for clinical services. This may explain the discrepancy between the Willcut (2012) finding and the current finding, since a large proportion of the current sample were recruited from the practices of clinicians. The low prevalence of ADHD hyperactive-impulsive type (only 1.7%) resulted in this category being excluded from further analysis.

Comparison of concordance and discordance rates in the group of sibling pairs where at least one member of each pair was classified as ADHD combined type, showed that the proportion of ADHD combined type concordant pairs was not statistically significantly different from the proportion of ADHD combined type / ADHD inattentive type discordant pairs. This result provides evidence for the ADHD combined subtype not “breeding true”, with relatives of ADHD combined type children not more likely to also have ADHD combined type rather than ADHD inattentive type. Although there was a significant difference in the ADHD inattentive type concordance versus ADHD inattentive type / ADHD combined type discordance rate in the group of siblings where at least one member of the sibling pair was classified as ADHD inattentive type, the difference was in the opposite direction than would be expected if ADHD inattentive type “bred true”. There was only one pair concordant for ADHD inattentive type, whilst all other pairs were discordant for ADHD inattentive type / ADHD combined type. As previously noted, these results point to ADHD combined type and ADHD inattentive type being varying presentations of the same disorder, rather than distinct disorders (Faraone, Biederman, & Friedman, 2000; Stawicki, Nigg, & Von Eye, 2006).

This finding is in line with findings from a number of previous family studies (Faraone,
When classifying subtypes according to the DSM-IV criteria, these researchers found no association between the subtype of a proband and that of his or her relatives. Two subsequent meta-analyses pooling data from multiple family studies for the most part also confirmed this finding (Stawicki, Nigg, & Von Eye, 2006; Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al., 2012). Although the meta-analysis by Stawicki, Nigg, & Von Eye (2006) found evidence for both subtype-specific and non-specific transmission for DSM-IV defined ADHD subtypes, the effect size for subtype-specific transmission was small. In the more recent meta-analysis, Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al. (2012) concluded that there is insufficient evidence to classify DSM-IV defined ADHD subgroups as distinct disorders.

Although more research is needed on the molecular and treatment response distinctions between the two subtypes (Willcutt, Nigg, Pennington, Solanto, Rohde, Tannock, et al., 2012), this result is also in line with findings from neurostructural, neurofunctional, and neurocognitive studies. Latter studies showed that ADHD inattentive type and ADHD combined type did show differences in white matter brain abnormalities, atypical neural connectivity, task related neurophysiological impairments, and neurocognitive deficits, but also shared neural abnormalities in all these domains (Fair, Nigg, Lyer, Bathula, Mills, Dosenbach, et al., 2013; Dovis, Van der Oord, Wiers, & Prins, 2015; Lei, Ma, Du, Shen, Jin, & Gong, 2014; Mazaheri, Fassbender, Coffey-Corina, Hartanto, Schweitzer, & Mangun, 2014).

5.5 Conclusion

In conclusion, the results from the current study in a South African sample of sibling pairs, concurs with results from previous family studies conducted in other countries. ADHD combined type and ADHD inattentive type do not seem to “breed true”, and thus are likely to represent varying presentations of the same underlying disorder, rather than distinct disorders. This study is the first of its kind in South Africa, and although the sample was not representative of the South African population, the findings and methods employed in this study may serve as a starting point, informing future studies in this country looking at the aetiological nature of the ADHD combined and ADHD inattentive subtypes. A better
understanding of the similarities and distinctions between the subtypes may in turn inform the design of molecular genetic studies into the differences between the two subtypes of ADHD in South Africa.

5.6 **Limitations of the study**

There were several important limitations to this study that should be noted. The sample being classified into sub-categories for the ADHD subtypes resulted in a relatively small number of participants in some of the categories, especially for the ADHD hyperactive-impulsive type classification. Future studies should aim to obtain a large enough sample so that all sub-diagnostic categories are well represented in the analysis. A further limitation was that this study relied on parent reported ADHD symptoms only for classification of subtypes. It has been shown that combining information from multiple informants improves the validity of ADHD diagnosis (Martel, Schimmack, Nikolas, & Nigg, 2015), and thus future studies should attempt to obtain symptom severity information from more than one source.

Although diagnostic classification was only used for descriptive purposes in this study, it should nonetheless be noted that parent-report of healthcare provider diagnosis was relied upon to classify children as ADHD or not ADHD. Structured diagnostic interviews directly performed by a healthcare provider would have been preferable, however, the time and costs involved in such a process was not feasible for this study. The decision to use parent-report of diagnosis was given some weight by a study conducted by Visser, Danielson, Bitsko, Perou, and Blumberg (2013) in which the prevalence rates of ADHD from parent-report of healthcare provider diagnosis was statistically indistinguishable from that gained directly from medical records of healthcare providers.

In addition, due to the exploratory nature of the study, exclusion criteria in the sample were relaxed. It is advisable that future studies apply more stringent exclusion criteria before attempting to replicate the results. Finally, due to the non-probability sampling process used, the sample was not representative of the South African population. Although this is common in behavioural genetic studies published in peer-reviewed journals (Agudelo, Gálvez, Fonesca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Van Dyk, Springer, Kidd, Steyn, Solomons, &
Van Toorn, 2014), it does prohibit the generalisation of the findings to the South African population at large. The findings in this study should therefore not be generalised to the South African population, but the methods employed should rather serve as a starting point for future research into this topic in South Africa.
Chapter 6

Examining the aetiology of the comorbidity of Attention-Deficit/Hyperactivity Disorder and Oppositional Defiant Disorder in a genetically informative sample\(^1\) from South Africa

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This chapter is the second chapter in the thesis to explore a possible reason for the mixed findings found in molecular genetic studies of Attention-Deficit/Hyperactivity Disorder (ADHD), namely the aetiological nature of the comorbidity between ADHD and ODD. Should ADHD plus ODD prove to be a distinct disorder from either ADHD or ODD, samples heterogeneous for these two forms of the disorder could result in conflicting findings. The research conducted, the statistical analysis, and the writing of this chapter was done by Nadia Fouché.

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\(^1\) A genetically informative sample is a sample which includes biological relatives.
Abstract

Attention-Deficit/Hyperactivity Disorder (ADHD) and Oppositional Defiant Disorder (ODD) are highly prevalent childhood externalising disorders that have been shown to frequently co-occur. Comorbid ADHD and ODD in children is associated with poorer outcomes compared to children diagnosed with only one of the disorders, making research into the aetiology of this comorbidity relevant and important. Although numerous studies have been conducted in this regard, findings have been inconclusive, with evidence for various mechanisms driving the comorbidity coming to the fore. In addition, no studies to date have been conducted in a sample from South Africa examining the frequency and aetiological nature of comorbid ADHD and ODD. This study aims to fill that gap by using a cross-sectional family study design in a South African sample. Four aetiological mechanisms proposed by previous authors are tested, namely ADHD and ODD co-occurring by chance only, comorbid ADHD and ODD representing an aetiologically distinct disorder, comorbid ADHD and ODD representing a more severe variant of ADHD, and ADHD and ODD co-occurring due to sharing causative environmental factors. The sample consisted of 164 siblings from 74 South African nuclear families, each with at least one child diagnosed with ADHD. The frequency of comorbid ADHD and ODD was comparable to that found in other countries. A multinomial logistic regression provided evidence for shared environmental factors influencing both disorders, driving their frequently reported co-occurrence. This finding may direct future research in South Africa to focus on factors in the environment when searching for aetiological factors in comorbid ADHD and ODD.

Keywords  Aetiological mechanisms, Childhood externalising disorders, Comorbidity, Family study, Multinomial logistic regression, Shared environmental factors
6.1 Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) and Oppositional Defiant Disorder (ODD) are two of the most frequently occurring childhood externalising disorders (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005). The world-wide prevalence of childhood ADHD is estimated at around 5% (American Psychiatric Association [APA], 2013; Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007), whilst the average prevalence of ODD is estimated at around 3.3% (APA, 2013). ADHD is characterised by a persistent pattern of inattention and/or hyperactivity-impulsivity (APA, 2013), and is associated with significant impairments in a number of domains for the diagnosed child, including difficulties with peers, academic problems, and family dysfunction (Foley, 2011; Hoza, 2007; Loe, & Feldman, 2007; Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012; Rubin, Bukowski, & Parker, 2007). ODD is defined in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as “a frequent and persistent pattern of angry/irritable mood, argumentative/defiant behaviour, or vindictiveness” (APA, 2013).

Similar to ADHD, ODD has its onset in early childhood (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005), and is also associated with both social impairment and family dysfunction (Greene, Biederman, Zerwas, Monuteaux, Goring, & Faraone, 2002). In addition, ODD has been found to precede conduct disorder in a number of studies, and also increases the risk for later anxiety disorders, mood disorders, and antisocial personality disorder (APA, 2013; Biederman, Petty, Monuteaux, Mick, Parcell, Westerberg, et al., 2008; Lavigne, Cicchetti, Gibbons, Binns, Larsen, & Devito, 2001; Rowe, Maughan, Pickles, Costello, & Angold, 2002).

Compounding the problem is the frequently replicated finding that ADHD and ODD co-occur more often than if by chance alone (Nock, Kazdin, Hiripi, & Kessler, 2007; Waschbusch, 2002). Studies from around the world have shown comorbidity rates of 28%, up to as high as 60% for ODD and ADHD (Biederman, 2005; Connor, Steeber, & McBurnett, 2010; Cuffe, Visser, Holbrook, Danielson, Geryk, Wolraich, et al., 2015; Inci, Ipci, Akyol Ardıç, & Ercan, 2016; Joelsson, Chudal, Gyllenberg, Kesti, Hinkka-Yli-Salomäki, Virtanen, et al., 2016). There is consistent evidence that the comorbid occurrence of ADHD and ODD is associated with poorer outcomes than if a child is diagnosed with only one of the two disorders (Connor, & Doerfler, 2008; Connor, Steeber, & McBurnett, 2010; Dalsgaard, Mortensen, Frydenberg, &
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Thomsen, 2002; Waschbusch, 2002). Furthermore, Connor, and Doerfler (2008) found that ADHD comorbid with ODD resulted in poorer educational outcomes, greater ADHD symptom severity, and greater levels of aggression and delinquency than either disorder diagnosed without the other. The high rates of comorbidity of these two disorders, along with the exacerbated negative outcomes compared to children only diagnosed with one of the two, have stimulated numerous research studies aiming to understand this comorbidity better (e.g. Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008; Harvey, Breaux, & Lugo-Candelas, 2016; Vierikko, Pulkkinen, Kaprio, & Rose, 2004; Zenglein, Schwenck, Westerwald, Schmidt, Beuth, Meyer, et al., 2016).

Regarding the aetiology of comorbid ADHD and ODD, a number of possible mechanisms underlying the frequent co-occurrence of these disorders have been put forth (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008; Hamshere, Langley, Martin, Agha, Stergiakouli, Anney, et al., 2013; Harvey, Breaux, & Lugo-Candelas, 2016; Tuvblad, Zheng, Raine, & Baker, 2009). Based on findings from previous research (Faraone, Biederman, Jetton, & Tsuang, 1997; Greene, Biederman, Zerwas, Monuteaux, Goring, & Faraone, 2002; Schachar, & Tannock, 1995), Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) proposed four possible mechanisms, as well as the expected outcomes for siblings of affected probands should each hold true, that can account for the co-occurrence of ADHD and ODD and/or conduct disorder (CD). The researchers used the broad term conduct problems (CP) to describe the occurrence of either CD or ODD.

Firstly, it is possible that ADHD and CP are etiologically independent of one another, and co-occur purely due to chance (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). Should this be the case, the recurrence risk for ADHD only, ADHD plus CP, and CP only in siblings of probands with ADHD plus CP should be low and more or less equal, whereas siblings of ADHD only probands will have a markedly higher risk of having only ADHD (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). However, as previously noted, the mere frequency with which ADHD and ODD co-occur makes this mechanism of comorbidity highly unlikely (Biederman, 2005; Connor, Steeber, & McBurnett, 2010; Waschbusch, 2002).
A second mechanism that has been proposed is that ADHD comorbid with CP is a qualitatively distinct disorder from either ADHD or CP occurring alone (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008; Hurtig, Ebeling, Taanila, Miettunen, Smalley, McGough, et al., 2007). Should this be the case, it would be expected that relatives of individuals diagnosed with ADHD and CP should also have higher rates of both ADHD and oppositional disorders, and not just higher rates of one of the disorders. In other words, the two disorders should co-segregate within families (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). Co-segregation is signalled if the co-occurring disorder in a proband occurs significantly more often in relatives who also have the other disorder, than in relatives without the other disorder (Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Geller, Petty, Vivas, Johnson, Pauls, & Biederman, 2007; Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al., 2009). Thus, in relatives of probands with both ADHD and ODD, co-segregation of the two disorders would be signalled if ODD occurred significantly more frequently in relatives who are also diagnosed with ADHD, than in those not diagnosed with ADHD. Furthermore, as noted by Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008), if ADHD plus CP is a distinct disorder, cases diagnosed with only ADHD will tend to have siblings who are also only diagnosed with ADHD. In contrast, cases diagnosed with ADHD comorbid with CP will tend to have siblings with ADHD and CP. Evidence for the co-segregation of ADHD and ODD in families has been found by both Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) and Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al. (2009).

In addition, Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) found that siblings of cases with ADHD plus CP were significantly more likely to also show symptoms of both ADHD and CP, compared to siblings of cases with only ADHD. Similarly, Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al. (2009) found that relatives of cases with comorbid ADHD and ODD, who were diagnosed with ADHD, were more likely to show symptoms of ODD than relatives not diagnosed with ADHD. That said, a comprehensive review by Waschbusch (2002) refuted the finding that ADHD plus CP is a separate taxonomic entity, noting that available evidence does not suggest that the combination of ADHD and conduct problems result in a separate disorder that is more than the sum of its parts.
The third mechanism proposed by Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) is that ADHD plus CP is a more severe variant of ADHD, with a greater genetic loading. Should this be the case, the two disorders would be expected to share a common genetic and environmental aetiology (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). Moreover, siblings of cases diagnosed with ADHD plus CP will be likely to have ADHD plus CP, but will also be likely to have only ADHD (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). Supporting this mechanism, Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) found that cases diagnosed with ADHD who had comorbid CP, had a nearly three-fold increased likelihood to have siblings who were only diagnosed with ADHD. In addition, a number of studies have found that ADHD and CP share both a common genetic and environmental aetiology (Thapar, Harrington, & McGuffin, 2001; Vierikko, Pulkkinen, Kaprio, & Rose, 2004), thus supporting the finding that ADHD plus CP is just a more severe form of the same disorder. That said, both Dick, Viken, Kaprio, Pulkkinen, and Rose (2005) and Tuvblad, Zheng, Raine, and Baker (2009), as well as Vierikko, Pulkkinen, Kaprio, and Rose (2004), found some degree of unique genetic influence for both ADHD and CP, whilst Tuvblad, Zheng, Raine, and Baker (2009) found the influence of shared environmental factors to be non-significant. These findings provide some credence for ADHD and ODD being separate disorders, and not just the quantitative extreme of a single disorder.

Finally, the fourth mechanism proposed by Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) for explaining the high rates of comorbidity between ADHD and CP, is that ADHD and CP share common environmental risk factors. If ADHD and CP are separate entities and co-occur due to shared environmental factors, siblings of probands diagnosed with ADHD only would be expected to have siblings with ADHD only. ADHD plus CP probands will have some siblings with ADHD only, and many siblings with ADHD plus CP and CP only (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). Previous studies have found evidence in support of the influence of shared environmental risk factors on the comorbidity between ADHD and ODD (Burt, Krueger, McGue, & Iacono, 2003, 2001; Burt, McGue, Krueger, & Iacono, 2005; Kuja-Halkola, Lichtenstein, D’Onofrio, & Larsson, 2015; Martin, Levy, Pieka, & Hay, 2006). However, it should be noted that all of these studies found genetic factors to also play a role in the co-occurrence of the disorders, and Kuja-Halkola, Lichtenstein, D’Onofrio, and Larsson (2015) found that stable shared environmental factors
contributed to the comorbidity, but only in early adolescence. Relating to the nature of the shared environmental factors influencing this comorbidity, Burt, Krueger, McGue, and Iacono (2003) found that parent-child conflict serves as a common risk factor, increasing the vulnerability for child externalising disorders. The authors posit that underlying pathological processes in the family environment drives the co-occurrence of these otherwise distinct psychological disorders. In contrast to these findings, numerous studies have found that the co-morbidity between ADHD and ODD are unlikely to be due to shared environmental influences (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Nadder, Rutter, Silberg, Maes, & Eaves, 2002; Tuvblad, Zheng, Raine, & Baker, 2009).

From the discussion above, it is evident that, apart from the first proposed mechanism where ADHD and ODD co-occur purely due to chance, all the other proposed mechanisms have some support in the literature. Further research is however needed to confirm the aetiological nature of the frequently reported comorbidity between ADHD and ODD. Furthermore, there has been no research on the comorbidity of ADHD and ODD thus far, nor of its aetiology, in a South African sample. Consequently, the main aim of the current study is to firstly investigate the frequency of comorbid ODD symptoms in a sample of ADHD diagnosed children in South Africa, and secondly, to use a family study design to test the above proposed mechanisms for this comorbidity by comparing results to the outcomes predicted by Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008). However, in contrast to the Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) study, the current study only focuses on symptoms of ODD (Table 6.1).
Table 6.1. Proposed mechanisms for comorbidity between ADHD and ODD.

<table>
<thead>
<tr>
<th>Proposed mechanism for comorbidity</th>
<th>Expected outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADHD plus ODD co-occur due to chance alone</strong></td>
<td>In siblings of cases with ADHD plus ODD</td>
</tr>
<tr>
<td></td>
<td>At equal risk for ADHD only, ADHD plus ODD and ODD only.</td>
</tr>
<tr>
<td></td>
<td>In siblings of cases with ADHD only</td>
</tr>
<tr>
<td></td>
<td>At risk for ADHD only.</td>
</tr>
<tr>
<td><strong>ADHD plus ODD is a distinct disorder</strong></td>
<td>In siblings of cases with ADHD only</td>
</tr>
<tr>
<td></td>
<td>At risk for ADHD only.</td>
</tr>
<tr>
<td><strong>ADHD plus ODD is a more severe variant</strong></td>
<td>In siblings of cases with ADHD only</td>
</tr>
<tr>
<td></td>
<td>At risk for ADHD only.</td>
</tr>
<tr>
<td><strong>ADHD plus ODD co-occur due to shared environmental factors influencing both disorders</strong></td>
<td>In siblings of cases with ADHD only</td>
</tr>
<tr>
<td></td>
<td>At risk for ADHD only, ADHD plus ODD and ODD only.</td>
</tr>
</tbody>
</table>

6.2 Methods

6.2.1 Participants

The sample consisted of a South African community sample of 164 children from 74 nuclear families, with 74 probands diagnosed with ADHD, and 90 siblings either diagnosed or unaffected. All nuclear families had to have had at least one child diagnosed with ADHD to be included in the sample. Diagnosis of ADHD was gleaned from parent-report of ADHD diagnosis by a healthcare professional. Children whose parents indicated that the child was diagnosed with ADHD by a healthcare professional were seen as having ADHD, whilst those who were indicated to not have been diagnosed were seen as being unaffected by the disorder. This methodology has been employed in previous studies (e.g. LeFever, Villers, & Morrow, 2002; Braun, Kahn, Froehlich, Auinger, & Lanphear, 2006) due to the considerable cost and time implications of having all participants directly diagnosed by a healthcare professional.

Only children between the ages of 5 and 18 years were included in the sample. Due to the design of the study being dependent on family members sharing genes, all adopted children were excluded from the sample. Due to the exploratory nature of the study, no
further exclusion criteria were applied. Of the 90 siblings, 27 were diagnosed with ADHD, whilst the remaining 63 were unaffected. ODD symptom severity, rather than a diagnostic classification of ODD as present/absent, was used in this study, and measured in all probands and siblings.

6.2.2 Procedure

Parents, who had at least one child diagnosed with ADHD, were recruited through social media pages via the Attention Deficit and Hyperactivity Support Group of Southern Africa (ADHASA), as well as through medical professionals working with children diagnosed with ADHD in the Bloemfontein area, Free State province of South Africa. Parents were provided with an information leaflet to read (Appendix A1), along with an informed consent form to sign (Appendix A2). After providing of consent to participate in the study, they were asked to complete the following measuring instruments:

(c) The SNAP-IV 26-item Teacher and Parent Rating Scale (Swanson, Kraemer, Hinshaw, Arnold, Conners, Abikoff, et al., 2001) (parent version) is based on the DSM-IV criteria for ADHD in children, covering both symptoms of inattention and hyperactivity/impulsivity (Appendix B2). A total score for ADHD symptom severity is derived from summing all items. The scale also includes items for measuring symptoms of ODD. The scale consists of 26 items, 18 measuring ADHD symptom severity, and eight measuring ODD symptom severity, all measured on a four-point Likert scale ranging from zero (Not at all) to three (Very much). Although the DSM-5 has been published, there are no essential differences in the symptoms listed in the DSM-IV (APA, 1994) and the DSM-5 (APA, 2013), and therefore the SNAP-IV scale was seen as appropriate for use in this study.

(d) A self-compiled questionnaire gathering biographical information such as gender and age, as well as a question related to whether the child has been diagnosed with ADHD or not (Appendix B1).

6.2.3 Statistical analysis

Data was cleaned prior to analysis and descriptive statistics calculated for all demographic and categorical variables. Mean imputation was used to replace missing values
in the SNAP-IV scale (Siddiqui, 2015). A parent of the participants had to have answered at least 15 out of the 18 questions (83%) measuring ADHD symptom severity, and six out of the eight questions measuring ODD symptom severity for means to be imputed. Any participants who answered fewer than 15 questions measuring ADHD symptom severity and fewer than six questions measuring ODD symptom severity were excluded from the inferential statistical analysis. To assure the reliability of the measuring instruments, internal consistency reliability was calculated for both the ADHD and ODD subscales of the SNAP-IV scale. Data analysis were conducted through the statistical software packages Stata version 14 (StataCorp, 2015) and SPSS version 23 (IBM Corp. Released 2013).

For hypothesis testing, probands and siblings were divided into groups according to their ADHD diagnoses and the severity of their ODD symptoms. ODD symptom severity was categorised according to the guidelines proposed by Swanson (n.d.): Scores < eight = “Symptoms not clinically significant”; scores from eight to 13 = “Mild symptoms”; scores from 14 to 18 = “Moderate symptoms”; and scores from 19 to 24 = “Severe symptoms”. Since all probands were diagnosed with ADHD, they were divided into two groups: probands with ODD scores greater than or equal to 14 were classified as “ADHD with moderate to severe ODD symptoms” (for brevity, henceforth referred to as “ADHD plus ODD”), and probands with ODD scores smaller than 14 were classified as “ADHD only”. Siblings were divided into four groups as follows: siblings not diagnosed with ADHD, but with ODD scores greater than or equal to 14, were classified as “No ADHD but moderate to severe ODD symptoms” (henceforth referred to as “ODD only”); siblings not diagnosed with ADHD and with ODD scores smaller than 14 were classified as “No ADHD or ODD”; siblings diagnosed with ADHD with ODD scores greater than or equal to 14 were classified as “ADHD with moderate to severe ODD symptoms” (henceforth referred to as “ADHD plus ODD”); and siblings diagnosed with ADHD with ODD scores smaller than 14 were classified as “ADHD only”. Subsequently, patterns of recurrence risk of ADHD and comorbid ODD in siblings of probands were analysed by means of a multinomial logistic regression. As was done by Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008), proband status as either “ADHD only” or “ADHD plus ODD” was used to predict sibling status in order to discern any salient patterns of recurrence risk in siblings of “ADHD only” or “ADHD plus ODD” probands. Since cases and their siblings were statistically not independent, and can thus be classified as related groups,
a Huber’s bootstrap correction (Huber, 1967) was implemented in STATA to control for correlated family data (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008; Faraone, Biederman, & Monuteaux, 2000). This formula produces robust statistical tests for logistic regression, and therefore makes assumption testing redundant (Faraone, Biederman, & Monuteaux, 2000). The significance level for all tests was set at an alpha value of 0.05, and all tests were two-sided (see Appendix E3 for extracts of the statistical analysis).

6.3 Results

6.3.1 Demographic characteristics

The average age for probands was 11 years, with 56 (75.7%) males and 18 (24.3%) females. For siblings, the average age was 10 years, with 48 (53.3%) males and 42 (46.7%) females (Table 6.2). The difference in age between the probands and the siblings was statistically significant (t = 2.419; p = 0.017), and there was a significant association between gender and participant classification as proband or sibling ($\chi^2 = 11.908; p = 0.001$).

<table>
<thead>
<tr>
<th></th>
<th>Probands</th>
<th>Siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ± SD)</td>
<td>11 ± 3</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (Count(%))</td>
<td>56 (75.7%)</td>
<td>48 (53.3%)</td>
</tr>
<tr>
<td>Female (Count(%))</td>
<td>18 (24.3%)</td>
<td>42 (46.7%)</td>
</tr>
</tbody>
</table>

6.3.2 Reliability of measuring instruments

The SNAP-IV scale showed good internal consistency reliability for both the ADHD and ODD subscales. For probands, Cronbach’s alpha coefficients of 0.916 and 0.914 were found for the ADHD and ODD subscales respectively. Results were similar for the siblings, with Cronbach’s alpha coefficients of 0.970 for the ADHD subscale and 0.928 for the ODD subscale.
6.3.3 Diagnostic status of siblings and probands

In the full sample of 164 children, 55 (33.5%) were diagnosed with ADHD without showing comorbid ODD symptoms, seven (4.3%) were not diagnosed with ADHD, but showed symptoms of ODD, and 46 (28.0%) were both diagnosed with ADHD and showed symptoms of ODD (Table 6.3).

<table>
<thead>
<tr>
<th></th>
<th>No ADHD or ODD</th>
<th>ADHD only</th>
<th>ODD only</th>
<th>ADHD plus ODD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probands</strong></td>
<td>0 (0%)</td>
<td>38 (51.4%)</td>
<td>0 (0%)</td>
<td>36 (48.6%)</td>
<td>74 (100%)</td>
</tr>
<tr>
<td><strong>Siblings</strong></td>
<td>56 (62.2%)</td>
<td>17 (18.9%)</td>
<td>7 (7.8%)</td>
<td>10 (11.1%)</td>
<td>90 (100%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>56 (34.1%)</td>
<td>55 (33.5%)</td>
<td>7 (4.3%)</td>
<td>46 (28.0%)</td>
<td>164 (100%)</td>
</tr>
</tbody>
</table>

6.3.4 Frequency of comorbidity between ADHD and symptoms of ODD

Descriptive statistics were run to determine the frequency of comorbid ODD symptoms in all children in the sample diagnosed with ADHD. Of the 101 children in the sample (including probands and siblings) diagnosed with ADHD, 46 (45.5%) children also showed moderate to severe ODD symptoms, whilst the remaining 55 (54.5%) children showed only mild or no ODD symptoms.

6.3.5 Multinomial logistic regression for testing patterns of recurrence risk in siblings

In line with the hypothesis set to be tested, siblings who were diagnosed with ADHD, but did not have moderate to severe ODD symptoms, were defined as the reference category for siblings (dependent variable) in the multinomial logistic regression. For probands (independent variable), ADHD only was defined as the reference category (Table 6.4).
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Table 6.4. Multinomial logistic regression results for patterns of recurrence risk in siblings.

<table>
<thead>
<tr>
<th>Dependent variable: Sibling ADHD/ODD status</th>
<th>Predictor variable: Proband ADHD/ODD status</th>
<th>Relative Risk Ratio (RRR)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ADHD or ODD</td>
<td>ADHD plus ODD</td>
<td>2.8</td>
<td>0.090</td>
</tr>
<tr>
<td>ADHD only</td>
<td>ADHD plus ODD</td>
<td>Reference category</td>
<td></td>
</tr>
<tr>
<td>ODD only</td>
<td>ADHD plus ODD</td>
<td>14.4*</td>
<td>0.031</td>
</tr>
<tr>
<td>ADHD plus ODD</td>
<td>ADHD plus ODD</td>
<td>2.4</td>
<td>0.249</td>
</tr>
</tbody>
</table>

*Relative risk ratio explained:
Risk for proband with ADHD plus ODD to have siblings with ODD only: 6/46 = 0.130434782. Risk for proband with ADHD plus ODD to have siblings with ADHD only: 5/46 = 0.108695652. Factor for difference in risk for ADHD plus ODD probands to have siblings with ODD only or ADHD only (Relative risk): 1.199999996.
Risk for proband with ADHD only to have siblings with ODD only: 1/44 = 0.022727272. Risk for proband with ADHD only to have siblings with ADHD only: 12/44 = 0.272727272. Factor for difference in risk for ADHD only probands to have siblings with ODD only or ADHD only (Relative risk): 0.08333333

Relative risk ratio for the difference between ADHD plus ODD cases and ADHD only cases in the difference in risk for probands to have siblings with ODD only or ADHD only: 1.199999996/0.08333333 = 14.40

The only finding that reached statistical significance in the model was for the difference in risk for ODD only versus ADHD only in siblings of probands diagnosed with ADHD plus ODD, compared to siblings of probands diagnosed with ADHD only. Results from the multinomial logistic regression model showed that siblings of cases with ADHD plus ODD have a significantly greater risk to have ODD only rather than ADHD symptoms only, than siblings of cases with ADHD only (RRR = 14.4; p = 0.031). More clarity of this result can be found by looking at the descriptive statistics of recurrence risk in siblings (Table 6.5).

Table 6.5. Recurrence risk in siblings of probands diagnosed with ADHD only and ADHD comorbid with symptoms of ODD.

<table>
<thead>
<tr>
<th>Proband</th>
<th>Sibling</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No disorder</td>
<td>ADHD only</td>
</tr>
<tr>
<td>ADHD only</td>
<td>26 (59.09%)</td>
<td>12 (27.27%)</td>
</tr>
<tr>
<td>ADHD plus ODD</td>
<td>30 (65.22%)</td>
<td>5 (10.87%)</td>
</tr>
<tr>
<td>Total</td>
<td>56 (62.22%)</td>
<td>17 (18.89%)</td>
</tr>
</tbody>
</table>

Of all siblings of probands who have ADHD plus ODD, 10.87% have been diagnosed with ADHD, but do not have moderate to severe ODD symptoms. In this same group of siblings, 13.04% showed moderate to severe ODD symptoms, but were not diagnosed with ADHD.
Thus, the risk was 1.2 times greater for siblings of probands diagnosed with ADHD plus ODD to have only ODD rather than only ADHD symptoms.

In comparison, when looking at siblings of probands who have ADHD, but do not have moderate to severe ODD symptoms (ADHD only), 27.27% have been diagnosed with ADHD, but do not have moderate to severe ODD symptoms (ADHD only). In this same group of siblings, only 2.27% showed moderate to severe ODD symptoms, but were not diagnosed with ADHD (ODD only). Thus, in the group of siblings for whom the corresponding probands were diagnosed with ADHD only, the risk was 12 times greater to have only ADHD symptoms rather than only ODD symptoms.

In summary, siblings of probands diagnosed with ADHD plus ODD were slightly more likely to have ODD only rather than ADHD only. In contrast, siblings of probands diagnosed with ADHD only were much more likely to have ADHD only rather than ODD only.

No further significant effects could be detected in model one. Of note is the finding that siblings of cases with ADHD plus ODD did not have a significantly greater risk to also have ADHD plus ODD, rather than ADHD only, compared to siblings of cases with ADHD only (RRR = 2.4; p = 0.249). Cases with ADHD only did have a greater likelihood to have siblings with ADHD only rather than siblings with ADHD plus ODD (27.3% versus 11.4%), but there was no difference in risk for siblings of cases with ADHD plus ODD (risk for ADHD only and ADHD plus ODD were both equal to 10.87%).

6.4 Discussion

This study aimed to determine the frequency of comorbid symptoms of ODD in children diagnosed with ADHD in a South African sample. In addition, this study aimed to test the hypotheses, put forth in the literature to explain the frequent co-occurrence of ADHD and ODD in children, by comparing results from a South African sample to the suggested outcomes for each hypothesis as set out by Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008).

In the current sample of 101 children diagnosed with ADHD, 45.5% showed comorbid moderate to severe ODD symptoms. This percentage falls within the 28% to 60% range found
in previous studies from around the world (Biederman, 2005; Connor, Steeber, & McBurnett, 2010; Cuffe, Visser, Holbrook, Danielson, Geryk, Wolraich, et al., 2015; Inci, Ipci, Akyol Ardiç, & Ercan, 2016; Joelsson, Chudal, Gyllenberg, Kesti, Hinkka-Yli-Salomäki, Virtanen, et al., 2016), and is the first estimate of comorbidity in a South African sample. It is important to note that due to the non-probability sampling procedure used, the sample was not representative of the South African population. Research on larger, more representative samples are recommended in South Africa to confirm this finding.

Recall that the only significant finding in the multinomial logistic regression showed a significant difference in the risk for ADHD only over ODD only between siblings of probands with ADHD plus ODD, and siblings of probands with ADHD only. Keeping this finding in mind, each of the posited hypotheses are discussed below in turn.

Testing hypothesis 1: ADHD plus ODD co-occur due to chance alone

Should hypothesis one prove to be true, siblings of probands with ADHD only would be expected to be at high risk for ADHD only, but would have no increased risk for ADHD plus ODD or ODD only. Siblings of probands with ADHD plus ODD would be expected to be at equal risk for ADHD only, ADHD plus ODD, and ODD only (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008).

This hypothesis is partly supported by the results of the multinomial logistic regression, since a significant difference in the risk for ADHD only over ODD only between siblings of probands with ADHD plus ODD, and siblings of probands with ADHD only, would be expected. In addition, the descriptive statistics did show equal risk of ADHD only, ODD only, and ADHD plus ODD for siblings of cases with ADHD plus ODD. However, although siblings of probands with ADHD only did have the greatest risk for ADHD only, compared to ODD only or ADHD plus ODD, they were also at risk for ADHD plus ODD, contrary to the expected results. The results thus only partially support this hypothesis. Together with the mere frequency of reported co-occurrence between the disorders (Biederman, 2005; Connor, Steeber, & McBurnett, 2010; Waschbusch, 2002), it is safe to assume that ADHD plus ODD do not co-occur due to chance alone.
Testing hypothesis 2: ADHD plus ODD as a distinct disorder

Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008) noted that if ADHD plus CP is a distinct disorder, separate from both ADHD and CP by themselves, children with ADHD only will have siblings who are at very high risk for also having ADHD only, whereas children with ADHD plus CP will have siblings at very high risk for ADHD plus CP. The results from the multinomial logistic regression did not support this hypothesis, indicating that siblings of probands with ADHD plus ODD did not have a significantly greater likelihood of being diagnosed with ADHD plus ODD rather than ADHD only, than siblings of probands with ADHD only. The descriptive statistics show that, although siblings of probands with ADHD only are more likely to have ADHD only rather than ADHD plus ODD, siblings of probands with ADHD plus ODD have an equal likelihood of having ADHD only and ADHD plus ODD. This finding is contradictory to that found by Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008), as well as Petty, Monuteaux, Mick, Hughes, Small, Faraone, et al. (2009), who found evidence for co-segregation of ADHD plus CP in siblings of ADHD plus CP probands, pointing to the possibility of ADHD plus CP being a distinct disorder. However, the results are in line with an earlier review by Waschbusch (2002), who concluded that the evidence available at that time was not sufficient to define ADHD plus CP as a separate taxonomic entity.

Testing hypothesis 3: ADHD plus ODD as a more severe variant of ADHD with a greater genetic loading (common genetic aetiology)

Should hypothesis three prove to be true, siblings of cases with ADHD plus ODD would be expected to have a high risk for ADHD only, and a very high risk for ADHD plus ODD, but no risk for ODD only. Siblings of cases with ADHD only would be expected to have an intermediate risk for ADHD only, and no risk for ADHD plus ODD or ODD only (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). Should this hypothesis be true, results from the multinomial logistic regression would not have shown a significant difference in risk for ADHD only over ODD only between siblings of cases diagnosed with ADHD only and ADHD plus ODD, since both groups of siblings would be expected to be at risk for ADHD only and not at risk for ODD only. The results from the multinomial logistic regression therefore do not support hypothesis three. The descriptive statistics confirm this finding. Siblings of
probands with ADHD only did have a high likelihood of also having ADHD only, but siblings of probands with ADHD plus ODD had risk percentages that were equally spread across ADHD only, ODD only, and ADHD plus ODD.

This finding again contradicts findings from Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al. (2008), who, along with finding evidence for ADHD plus CP being a distinct disorder, also found evidence for ADHD plus CP being a more severe disorder than ADHD only. That said, for ADHD plus ODD to be a more severe variant of ADHD, these two disorders would need to share a common genetic and environmental aetiology (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). There is, however, abundant evidence that this is not the case (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Tuvblad, Zheng, Raine, & Baker, 2009; Vierikko, Pulkkinen, Kaprio, & Rose, 2004), providing some support for the current finding.

**Testing hypothesis 4: ADHD plus ODD co-occur due to sharing environmental risk factors**

Should hypothesis four prove to be true, siblings of cases with ADHD plus ODD would be expected to have an intermediate risk for ADHD only, and a high risk for both ADHD plus ODD and ODD only. Siblings of cases with ADHD only would be expected to have a high risk for ADHD only, and no risk for ADHD plus ODD or ODD only (Christiansen, Chen, Oades, Asherson, Taylor, Lasky-Su, et al., 2008). Should this hypothesis be true, results from the multinomial logistic regression would be expected to show a significant difference in the risk for ADHD only over ODD only between siblings of probands with ADHD plus ODD, and siblings of probands with ADHD only, as shown in this equation:

\[
\text{Siblings of cases with ADHD plus ODD} = \text{Intermediate risk of ADHD only/High risk of ODD only} \\
\text{versus} \\
\text{Siblings of cases with ADHD only} = \text{High risk of ADHD only/No risk of ODD only}
\]

This hypothesis is supported by the current finding in the multinomial logistic regression of a significant difference in the risk for ADHD only over ODD only between siblings of probands with ADHD plus ODD, and siblings of probands with ADHD only. Also in line with this hypothesis, the descriptive statistics show that siblings of probands with ADHD only do have 12 times the risk of having ADHD only rather than ODD only, and more than twice the
risk to have ADHD only rather than ADHD plus ODD. Siblings of probands with ADHD plus ODD were approximately equally at risk for ADHD only, ODD only, and ADHD plus ODD. This finding is in line with previous findings showing a role for the shared environment in driving the comorbidity between ADHD and ODD (Burt, Krueger, McGue, & Iacono, 2003, 2001; Burt, McGue, Krueger, & Iacono, 2005; Kuja-Halkola, Lichtenstein, D’Onofrio, & Larsson, 2015; Martin, Levy, Pieka, & Hay, 2006). Although, of all these researchers, only Burt, Krueger, McGue, and Iacono (2001) found that shared environmental factors made the largest contribution to the comorbidity. These findings are in contrast to findings by Dick, Viken, Kaprio, Pulkkinen, and Rose (2005), Nadder, Rutter, Silberg, Maes, and Eaves (2002), and Tuvblad, Zheng, Raine, and Baker (2009), who found the influence of the shared environment on the comorbidity to be negligible.

6.5 Conclusion

In conclusion, similar to findings from samples from around the world, ADHD tended to frequently co-occur with symptoms of ODD in this South African sample, with nearly half of the children diagnosed with ADHD also showing moderate to severe ODD symptoms. In addition, our findings support the hypothesis that the comorbidity between ADHD and ODD is driven by the disorders sharing common environmental risk factors. The importance of the shared environment in the aetiology of ADHD, and now ODD, is once again highlighted.

6.6 Limitations of the study

Various limitations to this study should be noted. Firstly, due to the exploratory nature of the study, exclusion criteria for the sample were relaxed. Future studies in South Africa should consider applying more stringent exclusion criteria before aiming to replicate the findings. Secondly, the sample size was relatively small, especially with regards to the number of participants diagnosed with ODD only. Future studies should consider increasing the sample size to such an extent that all diagnostic subgroups are well represented.

Furthermore, for ODD symptom severity, this study made use of parent-report of symptoms only. Differences between parent and teacher reports of conduct problem symptoms have been previously reported (Papageorgiou, Kalyva, Dafoulis, & Vostanis, 2008). It is, therefore, advisable that future studies gain information regarding ODD symptom
severity across multiple informants or based on formal assessment by professionals. A further limitation was that the classification of children as being diagnosed with ADHD or not was based on parent-report of diagnosis by a healthcare professional. Diagnosis retrieved directly from healthcare professionals who conducted structured diagnostic interviews would have been preferable, but due to time and cost constraints, this was not feasible for the current study. The decision to use parent-report of healthcare provider diagnosis was supported by a study conducted by Visser, Danielson, Bitsko, Perou, and Blumberg (2013), in which the researchers reported no statistically significant difference in ADHD prevalence rates obtained from parent-report of healthcare provider diagnosis and prevalence rates gleaned directly from medical records of healthcare professionals.

A final limitation to this study was that, due to the non-probability sampling technique employed, the sample was not representative of the South African population. This is common in behavioural genetic research (e.g. Agudelo, Gálvez, Fonesca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Van Dyk, Springer, Kidd, Steyn, Solomons, & Van Toorn, 2014), and prohibits generalizations of the findings to the South African population at large. The statistical and family-study methodology employed can, however, inform future studies in South Africa conducted on samples representative of the general population.
This chapter on the sex differences in Attention-Deficit/Hyperactivity Disorder (ADHD) is a further attempt to show the heterogeneous nature of the disorder. Conflicting findings could result if the aetiology of the disorder is different for males and females, and if researchers do not distinguish between the sexes in their samples. The research conducted, the statistical analysis, and the writing of this chapter was done by Nadia Fouché.

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Abstract

Attention-Deficit/Hyperactivity Disorder is a highly prevalent childhood developmental disorder. The disorder is characterised by a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning in a number of domains. Research into the aetiology of the disorder is abundant, but is plagued by conflicting findings, which could possibly be due to heterogeneous subgroups existing within the population of diagnosed individuals. One possible factor dividing individuals into subgroups is gender, with the disorder being much more prevalent amongst males than females. Thus, the aim of this study was to test two models explaining the gender differences in ADHD, namely the polygenic multiple threshold model and the mean difference model. The sample consisted of 203 participants from 64 South African nuclear families. Through the use of analysis of covariance, controlling for age, it was determined that individuals from families with at least one female family member diagnosed with ADHD had higher ADHD symptom severity scores than individuals from families with only male members diagnosed with the disorder. This result provides evidence for the polygenic multiple threshold model, and against the mean difference model, suggesting that females have a higher diagnostic threshold for the disorder compared to males, and thus require a greater liability before being affected. This finding also provides further evidence for the existence of heterogeneous subgroups within the diagnosed population of individuals. The argument to identify these subgroups prior to conducting research on the disorder in order to limit conflicting findings is strengthened.

Keywords Analysis of covariance, Attention Deficit/Hyperactivity Disorder symptom severity, Family study, Gender, Mean difference model, Polygenic multiple threshold model
7.1 Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a childhood onset neurodevelopmental disorder, predominantly characterised by symptoms of inattention and/or hyperactivity-impulsivity which is severe enough to interfere with daily functioning and development (American Psychiatric Association [APA], 2013). With world-wide prevalence rates estimated at around 5% (Polanczyk, De Lima, Horta, Biederman, & Rohde, 2007), and Africa specific prevalence rates of 5% to 9% (Bakare, 2012), ADHD is viewed as one of the most prevalent neurodevelopmental disorders in children.

A multitude of studies have been conducted in an effort to determine the aetiology of ADHD, focusing on both putative genetic and environmental influences (Aguiar, Eubig, & Schantz, 2010; Banerjee, Middleton, & Faraone, 2007; Gizer, Ficks, & Waldman, 2009; Thapar, Cooper, Eyre, & Langley, 2013). Despite the large number of studies that have been conducted, the exact causes of ADHD are still far from understood. In particular, studies into the molecular genetics of the disorder have produced conflicting findings (Gizer, Ficks, & Waldman, 2009; Li, Chang, Zhang, Gao, & Wang, 2014; Sun, Yuan, Shen, Xiong, & Wu, 2013). Heterogeneity within the disorder is one possible reason for the conflicting results. There is abundant evidence that ADHD is an aetiologically heterogeneous disorder, with subgroups of patients differing in their disorder aetiology (Crosbie, & Schachar, 2001; Nigg, Willcutt, Doyle, & Sonuga-Barke, 2005; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015). Research samples consisting of a mix of these different subgroups of patients may be one of the reasons for the conflicting findings being reported, greatly hindering the search for specific aetiological factors influencing the disorder (Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al., 2014; Virkud, Todd, Abbacchi, Zhang, & Constantino, 2009). Before more research on the aetiology of the disorder is conducted, it would make sense to first identify these aetiologically distinct subgroups within the ADHD patient population, and determine what makes these subgroups distinct form one another. Studies on the aetiology of the disorder can then be conducted separately within each of the subgroups.

Two subgroups of patients that are perhaps the easiest to identify are those separated by sex, namely males and females. Apart from a number of differences in disorder
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presentation found between the sexes (Gershon & Gershon, 2002), one of the most consistent findings in the literature regarding ADHD is the difference in prevalence rates between males and females. It has been consistently found that ADHD is more prevalent in males than in females, with a ratio of between approximately 2:1 and 3:1 (APA, 2013; Ramtekkar, Reiersen, Todorov, & Todd, 2010; Willcutt, 2012).

Two closely related models, the polygenic multiple threshold model (PMT) (Rhee, & Waldman, 2004; Rhee, Waldman, Hay, & Levy, 1999) and the mean difference model (MDM) (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015), have been proposed to explain the differences in prevalence rates between males and females. The PMT model posits that multiple genetic and/or environmental factors influence a disorder such as ADHD in an additive fashion, and in so doing make up the liability to develop the disorder for a particular individual. Individuals in the population will differ in their disorder liabilities due to variable exposure to genetic and environmental factors. These differences in liabilities between individuals in a population or sub-population make up the population’s distribution of liability. Only individuals for whom the disorder liability exceeds a certain critical threshold will develop the disorder (Rhee, Waldman, Hay, & Levy, 1999). The PMT model is used to explain the difference in prevalence rates between males and females by postulating that multiple critical thresholds exist for different subgroups of the population. In the case of ADHD, the PMT model posits that females have a higher threshold for developing the disorder than males, and thus require a greater liability before manifesting the disorder (Rhee, & Waldman, 2004; Rhee, Waldman, Hay, & Levy, 1999). Should the PMT prove to be true, it would be expected that relatives of females who are diagnosed with ADHD will have more severe symptoms than relatives of males who are diagnosed. The reasoning behind this is that for females to be diagnosed, a greater disorder liability is required, which includes genetic liability. The relatives of a diagnosed female should thus also carry a greater liability than that of a diagnosed male, resulting in more severe symptoms of the disorder (Smalley, McGough, Del’Homme, NewDelman, Gordon, Kim et al., 2000).

Similarly, according to the MDM, the distribution of liability for females is shifted in the less-affected direction compared to that of males. Stated differently, males have a shifted distribution compared to that of females, with the mean for the males closer to the
diagnostic threshold. Thus, if the variances in distribution of liability for the male and female populations are equal, more males will fall into the affected tail of the distribution, and will more frequently be diagnosed with ADHD (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015). Should the MDM hold true, females diagnosed with ADHD should carry no greater liability for the disorder than males diagnosed with ADHD, and thus the relatives of diagnosed females should not display greater ADHD symptom severity than the relatives of diagnosed males. Since, according to the MDM, males in the population carry a greater liability for the disorder than females, males would be expected to have more severe ADHD scores than females (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015).

A number of studies have found evidence for the validity of the PMT model, showing that children with a maternal history of ADHD tended to have higher ADHD symptom severity than children with a paternal history of ADHD (Agha, Zammit, Thapar, & Langley, 2013; Goos, Ezzatian, & Schachar, 2007), and that siblings of girls with ADHD had a higher number of symptoms than siblings of boys with ADHD (Rhee, & Waldman, 2004; Rhee, Waldman, Hay, & Levy, 1999). Evidence supporting the MDM have also been found, with Ramtekkar, Reiersen, Todorov, and Todd (2010) and Arnett, Pennington, Willcutt, DeFries, and Olson (2015) reporting more severe inattention, hyperactivity-impulsivity, and total ADHD scores in males compared to females. Studies of this kind have, however, never been conducted in a South African sample. In addition, according to the researcher’s knowledge, no studies have yet been conducted comparing the closely related MDM and PMT models in their ability to explain the gender differences in the prevalence rates of ADHD.

Therefore, the aim of the current study is to test both the MDM and PMT models in a South African sample of nuclear families enriched for ADHD symptoms. It is hypothesised that, should the PMT model prove true, and the MDM model false, individuals from nuclear families with at least one diagnosed female will display more severe ADHD symptom scores than individuals from nuclear families with only diagnosed males. In contrast, no differences in ADHD symptom severity would be expected between individuals from nuclear families with males diagnosed compared to those with females diagnosed, should the MDM model prove true.
7.2 Methods

7.2.1 Participants

The sample consisted of a South African community sample of 203 participants from 64 nuclear families, enriched with children diagnosed with ADHD. Diagnosis of parental ADHD was gleaned from self-report of a diagnosis received by a healthcare professional by the parent. Classification of children as being affected by ADHD or not was made according to parent-report of diagnosis by a healthcare professional. This was in accordance with methodology employed in previous studies of ADHD (e.g. LeFever, Villers, & Morrow, 2002; Braun, Kahn, Froehlich, Auinger, & Lanphear, 2006), and was done in this manner due to the considerable time and cost implications of having all children in a sample formally assessed for ADHD directly by a healthcare professional. Thus, children who the parents indicated had received a diagnosis of ADHD by a healthcare professional were seen as being affected with the disorder, whilst children who parents indicated had not been diagnosed with ADHD by a healthcare professional were seen as not having ADHD.

Only children between the ages of 5 and 18 were included in the sample along with their parents. In addition, in order to ensure that all possible diagnosed females were accounted for in the nuclear families, only nuclear families where data from the mother was available were included in the sample. Due to the exploratory nature of the study, no further exclusion criteria were applied. There were 101 children, 64 mothers, and 38 fathers in the sample. Of the 101 children, 74 were diagnosed with ADHD, seven were suspected by their parents of having ADHD, and the remaining 20 were unaffected siblings. There were 95 individuals from families with at least one female diagnosed with ADHD, whilst 108 individuals had no females in their nuclear families diagnosed with ADHD. All participants were recruited through social media pages via the Attention Deficit and Hyperactivity Support Group of Southern Africa (ADHASA).

7.2.2 Procedures

Nuclear families who had at least one child either diagnosed with ADHD or strongly suspected of having ADHD, were recruited through ADHASA. Parents were provided with an information leaflet to read (Appendix A1), as well as an informed consent form to sign (Appendix A2). Hereafter they were asked to complete questionnaires measuring ADHD
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symptom severity for themselves, their child/children diagnosed with ADHD or suspected of having ADHD, as well as all unaffected siblings. The following measuring instruments were used:

(a) The SNAP-IV 26-item Teacher and Parent Rating Scale (Swanson, Kraemer, Hinshaw, Arnold, Conners, Abikoff, et al., 2001) (parent version) is based on the DSM-IV criteria for ADHD in children, covering both symptoms of inattention and hyperactivity-impulsivity (Appendix B2). Although a newer version of the DSM has been published (DSM-5), the symptoms listed for ADHD are the same between the two versions (DSM-IV and DSM-5), and therefore the SNAP-IV scale was seen as an appropriate instrument to use (APA, 1994; APA, 2013). A total score for ADHD symptom severity is derived from summing all items. The scale also includes items for measuring symptoms of Oppositional Defiant Disorder (ODD), but these items were not analysed in this chapter. The scale consists of 18 items measuring ADHD symptom severity, all measured on a four-point Likert scale ranging from zero (Not at all) to three (Very much).

(b) The Adult ADHD Self-Report Scale (ASRSv1.1) Symptom Checklist (Kessler, Adler, Ames, Demler, Faraone, Hiripi, et al., 2005) is a self-assessment measure developed by the World Health Organisation (WHO) for the screening of ADHD in adults (Kessler, Adler, Ames, Demler, Faraone, Hiripi, et al., 2005) (Appendix B4). Similar to the SNAP-IV, the items on the ASRS were derived from the DSM-IV criteria for ADHD diagnoses, but item wording was modified to be applicable to adults. The scale consists of 18 items measuring ADHD symptom severity, each rated on a five-point Likert scale ranging from 0 (Never) to 4 (Very often).

7.2.3 Statistical analysis

Descriptive statistics were calculated for all demographic variables. In addition, internal consistency reliability coefficients were calculated for total scale scores of the SNAP-IV and ASRS measuring instruments. Mean imputation was used to replace missing values in the SNAP-IV and ASRS scales (Siddiqui, 2015). For the SNAP-IV scale, a parent had to have answered at least 15 out of the 18 (83%) questions for a child for means to be imputed. For
the ASRS scale, parents also had to have answered 15 out of the 18 (83%) questions for themselves for means to be imputed. Answering fewer than 15 out of the 18 questions for either the SNAP-IV scale or the ASRS resulted in participants being excluded from the inferential statistical analysis. Scores from the SNAP-IV and ASRS were standardised in order to ensure comparability prior to analysis. In addition, due to the wide age range of the sample, it was decided to control for the effects of age in the analysis. Due to the clustered nature of the data (individuals were clustered within families) and the subsequent possibility of non-independence between members from the same family, the need to conduct multilevel modelling was first tested by determining whether there was any clustering of data within families. Firstly, a model was built consisting only of the level one variable, namely whether an individual was from a family with a female diagnosed with ADHD (henceforth referred to as the Female Diagnosed variable), and the outcome variable (ADHD symptom severity scores), not taking the influence of family structure into account. Secondly, both the Female Diagnosed variable and age were entered as fixed effects, with age entered as a covariate and Female Diagnosed entered as a factor. Age was grand-mean centred prior to entry into the model as is common practice in multilevel models (Field, 2013). Hereafter, a second model was constructed that did take family structure into account. This was done by adding the level two variable of belonging to a specific family as a random effect with varying intercepts to the first model. Finally, a comparison of these two models was conducted through the comparison of the $-2$ Log Likelihood values in order to determine whether factoring in the clustering of individuals within families made a significant improvement in the fit of the model. The $-2$ Log Likelihood values were compared by means of a chi-square test of statistical significance. A significant chi-square test would indicate a significant improvement in the model, and thus a significant influence of the contextual variable. This would in turn be indicative of the need to use multilevel modelling for further analysis. Should the addition of the contextual variable not result in a significant improvement in the model, an analysis of covariance (ANCOVA) would be conducted to determine whether there is a significant difference in the mean ADHD symptom severity scores between individuals from families with at least one female diagnosed with ADHD, and those with no female family members diagnosed with the disorder, after controlling for the effects of age.
The significance level for the tests were set at an alpha value of 0.05, and all tests were two-sided (see Appendix E4 for extracts of the statistical analysis).

7.3 Results
7.3.1 Demographic characteristics

The majority of participants were white (92.6%) and either Afrikaans or English speaking (96.6%), and thus not representative of the South African population. Amongst the children, there were more males than females in the sample. However, more mothers completed the survey than fathers (Table 7.1).

7.3.2 Reliability of measuring instruments

The SNAP-IV total scale has been found to have good internal consistency reliability, with an alpha coefficient of 0.94 in a United States of America sample of elementary school children (Bussing, Fernandez, Harwood, Wei, Garvan, Eyberg et al., 2008). Studies in South Africa looking at the internal consistency of the scale are lacking. In this study, the total scale of the SNAP-IV also proved to have high internal consistency reliability, with a Cronbach’s alpha coefficient of 0.963.

Unlike SNAP-IV, a study has been done in South Africa in which the internal consistency reliability of the ASRS total scale has been reported (Burke, Austin, & Waldeck, 2011). These researchers found adequate internal consistency reliability for the ASRS, with a Cronbach’s alpha value of 0.886 reported for the total scale. In the current study, high internal consistency reliability for the ASRS was also found, with a Cronbach’s alpha coefficient of 0.954 for the total scale.
Table 7.1. Demographic characteristics of the sample.

<table>
<thead>
<tr>
<th></th>
<th>Fathers</th>
<th>Mothers</th>
<th>Sons</th>
<th>Daughters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of individuals</strong></td>
<td>38</td>
<td>64</td>
<td>73</td>
<td>28</td>
</tr>
<tr>
<td><strong>Age (Mean ± SD)</strong></td>
<td>42 ± 7</td>
<td>38 ± 5</td>
<td>9 ± 3</td>
<td>10 ± 3</td>
</tr>
<tr>
<td><strong>Home language</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afrikaans (Count(%))</td>
<td>15 (39.5%)</td>
<td>22 (34.4%)</td>
<td>25 (34.2%)</td>
<td>9 (32.1%)</td>
</tr>
<tr>
<td>English (Count(%))</td>
<td>16 (42.1%)</td>
<td>34 (53.1%)</td>
<td>39 (53.4%)</td>
<td>15 (53.6%)</td>
</tr>
<tr>
<td>Afrikaans and English</td>
<td>6 (15.8%)</td>
<td>6 (9.4%)</td>
<td>7 (9.6%)</td>
<td>2 (7.1%)</td>
</tr>
<tr>
<td>(Count(%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sesotho (Count(%))</td>
<td>0 (0%)</td>
<td>1 (1.6%)</td>
<td>2 (2.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Home language not indicated</td>
<td>1 (2.6%)</td>
<td>1 (1.6%)</td>
<td>0 (0.0%)</td>
<td>2 (7.1%)</td>
</tr>
<tr>
<td>(Count(%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White (Count(%))</td>
<td>35 (92.1%)</td>
<td>59 (92.2%)</td>
<td>68 (93.2%)</td>
<td>26 (92.9%)</td>
</tr>
<tr>
<td>Black (Count(%))</td>
<td>0 (0%)</td>
<td>1 (1.6%)</td>
<td>2 (2.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Coloured (Count(%))</td>
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<td>1 (1.6%)</td>
<td>1 (1.4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Indian (Count(%))</td>
<td>1 (2.6%)</td>
<td>2 (3.1%)</td>
<td>2 (2.7%)</td>
<td>1 (3.6%)</td>
</tr>
<tr>
<td>Race not indicated</td>
<td>1 (2.6%)</td>
<td>1 (1.6%)</td>
<td>0 (0.0%)</td>
<td>1 (3.6%)</td>
</tr>
<tr>
<td>(Count(%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7.3.3 Comparison of the multilevel models

The variables entered in each of the multilevel models that were compared in order to determine whether the clustering of individuals within families had a significant influence and thus needed to be factored into the analysis, can be seen below (Table 7.2).

The results for the comparison of the two multilevel models are presented below for the total ADHD symptom domain (Table 7.3). As described under statistical techniques, for model one, only the Female Diagnosed variable and age were entered without taking family structure into account. Model two consisted of the same variables as model one, but this time family structure was taken into account.
Sex differences in the prevalence of ADHD / 135

Table 7.2. Variables entered in the multilevel models to be compared.

<table>
<thead>
<tr>
<th></th>
<th>Total ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
</tr>
<tr>
<td><strong>Fixed effects</strong></td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Diagnosed</td>
</tr>
<tr>
<td></td>
<td>Age</td>
</tr>
<tr>
<td><strong>Random effects</strong></td>
<td>None</td>
</tr>
</tbody>
</table>

Table 7.3. Results of model comparison for having a female member in the family diagnosed with ADHD as predictor of total ADHD symptom severity, adjusted for age.

<table>
<thead>
<tr>
<th></th>
<th>Minus 2 Log Likelihood value for Model 1</th>
<th>Minus 2 Log Likelihood value for Model 2</th>
<th>Chi-square change</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD symptom severity</td>
<td>560.74</td>
<td>560.07</td>
<td>0.67</td>
<td>1</td>
</tr>
</tbody>
</table>

*p<0.05 (Critical value for Chi-square statistic at 1 degree of freedom = 3.84)

The addition of family structure did not result in a significant improvement in model fit for ADHD symptom severity (p>0.05) (Table 7.3). It can consequently be concluded that the clustering of individuals within families did not have a significant impact on the analysis. For this reason, multilevel modelling was not used for any further analysis.

7.3.4 Analysis of covariance

An analysis of covariance (ANCOVA) was run to determine the effect of being from a nuclear family where at least one female family member has been diagnosed with ADHD on ADHD symptom severity scores, after controlling for age. There was a linear relationship between age and ADHD symptom severity scores for both individuals with a female family member diagnosed with ADHD, and those without, as assessed by visual inspection of a scatterplot. There was homogeneity of regression slopes as the interaction term was not statistically significant, F(1,198) = 0.001, p = 0.971. Standardised residuals for the two groups
of the independent variable and for the overall model were normally distributed, as assessed by Normal Q-Q Plots. There was homoscedasticity and homogeneity of variances, as assessed by visual inspection of a scatterplot and Levene’s test of homogeneity of variance (p = 0.677), respectively. There were no outliers in the data, as assessed by no cases with standardised residuals greater than ±3 standard deviations. After adjustment for age, there was a statistically significant difference in ADHD symptom severity scores between individuals with at least one female nuclear family member diagnosed with ADHD and those with only male family members diagnosed, F(1,199) = 11.623, p < 0.05, partial η2 = 0.055. Individuals with a female family member diagnosed with ADHD in their nuclear family had significantly higher mean ADHD symptom severity scores than individuals from families with only males diagnosed (see Table 7.4).

Table 7.4. Adjusted and unadjusted means and variability for ADHD symptom severity* within the groups of the independent variable, with age as a covariate.

<table>
<thead>
<tr>
<th>Female in family diagnosed</th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Mean</td>
</tr>
<tr>
<td>Yes</td>
<td>94</td>
<td>0.25</td>
<td>0.999</td>
<td>0.25</td>
</tr>
<tr>
<td>No</td>
<td>108</td>
<td>-0.22</td>
<td>0.953</td>
<td>-0.22</td>
</tr>
</tbody>
</table>

*ADHD symptom severity scores reflect standardised Z-scores

7.4 Discussion

Cronbach’s alpha coefficients for the two measuring instruments, the SNAP-IV and ASRS, were of adequate magnitude, and comparable to that reported by previous studies. It can thus be concluded that these measuring instruments were sufficiently reliable for use in the current study. Descriptive statistics showed that the sample was not representative of the South African population. This was expected, since a non-probability sampling strategy was used due to cost and time constraints. Although non-representative samples is common in published studies in behavioural genetics research (Agudelo, Gálvez, Fonesca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Van Dyk, Springer, Kidd, Steyn, Solomons, & Van Toorn, 2014),
this does preclude generalising the results of this study to the South African population at large.

As previously mentioned, the PMT model, as an explanation of gender differences in the prevalence of ADHD, posits that females have a higher threshold for the disorder compared to males, and thus need to carry a greater liability before manifesting symptoms (Rhee, & Waldman, 2004; Rhee, Waldman, Hay, & Levy, 1999). Alternatively, the MDM model explains the gender differences in occurrence rates by postulating that males have a shifted distribution in liability compared to females, with the male mean falling closer to the diagnostic threshold. If equal variances are assumed, the result would be that more males will have liabilities exceeding the diagnostic threshold compared to females (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015).

Similar to the findings of previous studies (Agha, Zammit, Thapar, & Langley, 2013; Goos, Ezzatian, & Schachar, 2007; Rhee, & Waldman, 2004; Rhee, Waldman, Hay, & Levy, 1999), the results of the current study support the PMT model. Individuals with at least one female in their nuclear family diagnosed with ADHD showed greater ADHD symptom severity scores than individuals with only male family members diagnosed with ADHD. This finding is in line with the PMT model, since if females do need a greater liability for the disorder before expressing symptoms, it would be expected that diagnosed females carry a higher liability than diagnosed males. Since a proportion of this liability is genetic, it would be expected that individuals from nuclear families with at least one diagnosed female would also carry this greater liability, and consequently display more severe symptoms than individuals from families with only diagnosed males (Smalley, McGough, Del'Homme, NewDelman, Gordon, Kim et al., 2000).

In contrast, unlike the findings by Ramtekkar, Reiersen, Todorov, and Todd (2010) and Arnett et al. (2015), the findings of the current study do not support the MDM model, since a shifted distribution of liability in the female population relative to that of males away from the diagnostic threshold would not result in diagnosed females carrying a higher liability for the disorder compared to diagnosed males. The diagnostic threshold would stay the same for males and females. Thus, unlike the findings of the current study, no differences in symptom severity would be expected between individuals with at least one female family
member diagnosed with ADHD and those with only males diagnosed (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015).

7.5 Conclusion

The finding in this study that individuals from nuclear families with at least one female diagnosed with ADHD had more severe ADHD symptom scores than individuals from families with only males diagnosed provides support for the PMT model. It is thus possible that females do indeed have a higher threshold for the disorder compared to males, and need a greater liability before developing the disorder. Support was not provided for the MDM model, suggesting that females and males do not have an equivalent threshold for the disorder with females’ liability distribution just shifted away towards the unaffected side of the distribution. This provides further evidence of the heterogeneous nature of the disorder, and the need to identify subgroups of patients prior to undertaking research. Moreover, conducting research in these identified subgroups may limit the conflicting findings plaguing research into the aetiology of ADHD to date.

7.6 Limitations of the study

Due to this study being the first of its kind in a South African sample of nuclear families, the only exclusion criteria that were stringently applied were that children in the sample needed to be between the ages of 5 and 18 years, and that data needed to be available for all mothers in the nuclear families. Future studies may need to apply more stringent exclusion criteria. Furthermore, the nuclear family structure of the sample resulted in individuals’ ages varying extensively. This necessitated the use of adult-specific and child-specific measuring instruments of ADHD symptom severity. Although the measuring instruments measured the same symptom dimensions, and scores were standardised in order to be comparable, future studies may consider using samples more homogeneous in age prior to trying to replicate these findings.

Due to the non-probability sampling strategy employed, the sample in this study was not representative of the South African population, and results from this study should therefore not be generalised to the South African population at large. As mentioned, this is common in published studies in the field of behavioural genetics. The statistical and family-
study methods employed can however inform future studies on representative samples in South Africa aimed at explaining sex differences in ADHD. A further limitation to this study was that, due to time and cost constraints, participants were not all assessed directly for ADHD by a healthcare provider. Rather, parent- and self-report of ADHD diagnosis by a healthcare provider was relied upon for classification of participants as being affected with ADHD or not. It is therefore possible that participants who were classified as “unaffected” had undiagnosed ADHD. However, lending support to the methodology employed here is a study by Visser, Danielson, Bitsko, Perou, and Blumberg (2013) in which it was found that the prevalence of parent-report of ADHD diagnosed by a healthcare provider was statistically indistinguishable from the prevalence of ADHD diagnosis gleaned directly from medical records of healthcare providers.
Chapter 8

Influence of pregnancy and delivery complications on ADHD symptom severity in children

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In this chapter, the heterogenous nature of Attention-Deficit/Hyperactivity Disorder (ADHD) is further explored through looking at the differential impact of pregnancy and delivery complications on ADHD symptom severity in simplex versus multiplex ADHD families. Should the simplex/multiplex distinction in ADHD prove to be valid, studies examining the aetiology of ADHD without distinguishing between simplex and multiplex ADHD in their samples may yield conflicting findings. The research conducted, the statistical analysis, and the writing of this chapter was done by Nadia Fouché.

Acknowledgements: Financial support by the National Research Foundation, South Africa, is greatly acknowledged.
Abstract

Studies into the genetic aetiology of Attention-Deficit/Hyperactivity Disorder (ADHD) have yielded conflicting findings. An explanation for the conflicting findings is that simplex and multiplex forms of the disorder exist, with different aetiological factors influencing each. In research on the simplex/multiplex distinction in Autism Spectrum Disorder, the simplex form of the disorder was only influenced by non-shared genetic and environmental factors, whilst the multiplex form was influenced by polygenic and/or shared environmental factors. Environmental factors influencing ADHD have mostly been shown to be associated with pregnancy and/or delivery complications. The aim of this study was to firstly determine the shared or non-shared nature of pregnancy and delivery complications. Secondly, the hypothesis was tested that simplex and multiplex ADHD are aetologically distinct, with simplex ADHD being influenced by environmental factors unique to the individual, whilst multiplex ADHD is influenced by shared environmental factors. In a South African community sample of 171 siblings from 83 nuclear families, results showed that pregnancy complications were both shared and non-shared in nature. Delivery complications were found to only be non-shared. Through generalised estimating equations modelling, pregnancy complications were found not to be associated with ADHD symptom severity. Delivery complications were associated with hyperactivity and inattention symptom severity. The simplex/multiplex distinction only played a role for the effect of delivery complications on inattention symptom severity, with the complications only having an impact on inattention symptoms in simplex ADHD families. This result provides evidence for the existence of simplex and multiplex forms of the inattention symptom domain of ADHD, with only the simplex form being influenced by non-shared environmental factors.

Keywords Generalised estimating equations modelling, Hyperactivity-impulsivity, Inattention, Pregnancy and delivery complications, Shared and non-shared aetiological factors, Simplex and multiplex ADHD
8.1 Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a highly prevalent childhood-onset disorder, with a world-wide pooled prevalence estimated at 5% for subjects 18 years or younger (Polanczyk, De Lima, Horta, Biederman, & Rohde, 2007). The core features of the disorder include developmentally inappropriate symptoms of inattention and/or hyperactivity-impulsivity that interferes with functioning or development. In the latest revision of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) (5th ed.; DSM–5; American Psychiatric Association [APA], 2013), three main forms of presentation of the disorder are specified, namely:

➢ Combined presentation, characterised by the presence of both inattention and hyperactivity-impulsivity symptoms;
➢ Predominantly inattentive presentation, characterised by the presence of predominantly inattentive symptoms; and
➢ Predominantly hyperactive-impulsive presentation, characterised by the presence of mainly hyperactive-impulsive symptoms (APA, 2013).

Although consensus has been reached that ADHD is a highly heritable disorder [a meta-analysis by Nikolas and Burt (2010) found heritability estimates of above 70% for both the hyperactivity and inattention dimensions], there is a great degree of conflicting findings in molecular genetic studies of the disorder (Gizer, Ficks, & Waldman, 2009; Li, Chang, Zhang, Gao, & Wang, 2014; Sun, Yuan, Shen, Xiong, & Wu, 2013). In non-Mendelian disorders like ADHD, various factors may obscure the findings of genetic studies. Amongst these are aetiological heterogeneity, reduced penetrance, variable expressivity, and incorrectly defining the behavioural phenotype (Crosbie, & Schachar, 2001). In particular, evidence abound that ADHD is an aetiologically heterogeneous disorder, with aetiological mechanisms varying for different subgroups of patients (Crosbie & Schachar, 2001; Nigg, Willcutt, Doyle, & Sonuga-Barke, 2005; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015). Samples consisting of a mix of aetiologically heterogeneous subgroups may greatly hinder the search for specific aetiological factors influencing the disorder, resulting in conflicting findings between different sampled populations (Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar et al., 2014; Virkud, Todd, Abbacchi, Zhang, & Constantino, 2009).
Thus, finding ways to decrease heterogeneity in samples of ADHD children may be imperative to reduce conflicting findings in the search for causes of the disorder going forward.

One promising avenue that has been explored extensively in research on Autism Spectrum Disorders (ASD), but is still very much unexplored in ADHD research, is the distinction between simplex and multiplex families (Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar et al., 2014; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015; Sullivan, Daly, & O’Donovan, 2012). Simplex families refer to families with only one affected family member, whereas in multiplex families, at least two family members are affected by a disorder (Oerlemans, Burmanje, Franke, Buitelaar, Hartman, & Rommelse, 2016; Sullivan, Daly, & O’Donovan, 2012). Research on ASD has found that causal factors differ between the simplex and multiplex forms of the disorder, with simplex ASD being influenced to a greater degree by risk factors unique to the individual, whereas multiplex ASD was influenced to a greater extent by factors shared between multiple family members. Thus, the simplex/multiplex design, if applicable, enables researchers to study sample populations that are either enriched for individual specific genetic and/or environmental causes (i.e. in samples from simplex families), or sample populations enriched for polygenic and/or shared environmental causes (in samples from multiplex families) (Freitag, 2007; Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al., 2014; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015; Oerlemans, Burmanje, Franke, Buitelaar, Hartman, & Rommelse, 2016; Sebat, Lakshmi, Malhotra, Troge, Lese-Martin, Walsh, et al., 2007; Sullivan, Daly, & O’Donovan, 2012).

The few studies that have looked at the usefulness of the simplex/multiplex family distinction in ADHD have reported conflicting results. Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al. (2014) compared behavioural traits associated with ADHD between unaffected family members in simplex versus multiplex ADHD families, as well as control families. The assumption was that if ADHD in the multiplex families was caused by different factors than that in the simplex families (shared environmental/polygenetic factors versus individual specific genetic/environmental factors), unaffected members from the two types of families would differ in the ADHD symptoms they show. More specifically, unaffected members in multiplex families were hypothesised to show a greater degree of ADHD
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symptomology than unaffected members in simplex families. Results from this study showed, however, that unaffected members from both simplex and multiplex ADHD families showed equally elevated levels of ADHD symptoms compared to members from the control families, pointing to ADHD possibly only being influenced by shared environmental and/or polygenetic factors shared between family members.

However, in a similarly designed study, Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, and Rommelse (2015) looked at an intermediate phenotype instead of ADHD traits directly. In contrast to the previous finding, the authors found that cognitive vulnerability profiles (as an endophenotype of ADHD) did indeed differ between simplex, multiplex, and control families. In the multiplex ADHD families, unaffected members showed a cognitive vulnerability profile intermediate between that shown by affected members and members from control families. In contrast, unaffected members from simplex ADHD families for the most part had a cognitive vulnerability profile indistinguishable from that of controls. This result supports the hypothesis that simplex ADHD is influenced by factors specific to the individual, with no influence on other family members, whereas multiplex ADHD is influenced by factors shared between family members, resulting in all family members showing symptoms. Therefore, this result points to the possibility of different aetiological factors influencing ADHD in simplex versus multiplex families.

Recent evidence supports the above finding and indeed points to rare genetic variants unique to the individual, as well as individual-specific environmental factors having an influence on ADHD. De novo rare copy number variants (CNV’s) have been identified in ADHD cases, as well as in children displaying attention problems (Ehli, Abdellaoui, Hu, Hottenga, Kattenberg, Beijsterveldt, et al., 2012; Lionel, Crosbie, Barbosa, Goodale, Thiruvahindrapuram, Rickaby, et al., 2011). Moreover, environmental risk factors influencing ADHD have been found to be mostly related to factors surrounding pregnancy and birth (Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al., 2012; Oerlemans, Burmanje, Franke, Buitelaar, Hartman, & Rommelse, 2016). That said, apart from the well documented detrimental effects of maternal smoking and alcohol consumption during pregnancy on ADHD symptomology (Bhatara, Loudenberg, & Ellis, 2006; Coles, Platzman, Lynch, & Freides, 2002; Fryer, McGee, Matt, Riley, & Mattson, 2007; Gustafsson, & Källén, 2011; Knopik,
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Sparrow, Madden, Bucholz, Hudziak, Reich, et al., 2005; Nomura, Marks, & Halperin, 2010; findings regarding an association between other pregnancy and delivery complications and ADHD have been conflicting (Buschgens, Swinkels, Van Aken, Ormel, Verhulst, & Buitelaar, 2009; Ketzer, Gallois, Martinez, Rohde, & Schmitz, 2012; St Sauver, Barbaresi, Katusic, Colligan, Weaver, & Jacobsen, 2004; Wagner, Schmidt, Lemery-Chalfant, Leavitt, & Goldsmith, 2009). Factors investigated by these researchers included induction or augmentation of labour, breech presentation, abnormal fetal positions, mode of delivery, lack of oxygen, blood transfusion, jaundice, any operative procedures, hypertension, gestational diabetes, anaesthesia, pre-eclampsia, placental abnormalities, chronic maternal disease, and bleeding. Buschgens, Swinkels, Van Aken, Ormel, Verhulst, and Buitelaar (2009) and Ketzer, Gallois, Martinez, Rohde, and Schmitz (2012) found significant associations between pregnancy and delivery complications and ADHD, but only for the inattention symptom dimension. In contrast, St Sauver, Barbaresi, Katusic, Colligan, Weaver, and Jacobsen (2004) and Wagner, Schmidt, Lemery-Chalfant, Leavitt, and Goldsmith (2009) found no significant association between pregnancy and delivery complications and ADHD.

The simplex/multiplex distinction in ADHD might provide a possible explanation for the conflicting results regarding the influence of pregnancy and delivery complications. Amor, Grizenko, Schwartz, Lageix, Baron, Ter-Stepanian, et al. (2005) proposed that some of the pregnancy and delivery complications may represent shared environmental risk factors, whereas others are of a non-shared nature. Should the simplex/multiplex distinction prove to be true, the non-shared pregnancy and delivery complications will be expected to play a role only in simplex ADHD families, whereas the effects of shared pregnancy and delivery complications will be evident only in multiplex ADHD families. Not stratifying samples into multiplex and simplex ADHD would then result in conflicting findings regarding the role of these risk factors.

The first study to partition pregnancy and delivery complications into shared and non-shared environmental factors, and divide ADHD families into simplex and multiplex families was recently conducted by Oerlemans, Burmanje, Franke, Buitelaar, Hartman, and Rommelse (2016). Findings from this study showed that the simplex/multiplex distinction in ADHD provided no further insight into the role of shared and non-shared pregnancy and
delivery complications in ADHD. That said, the latter study looked at ADHD as a categorical diagnosis only. In Chapter 3 of this thesis, it was shown that inattention symptoms seem to cluster in families, but not hyperactivity-impulsivity symptoms. Given that this finding in Chapter 3 provides evidence for the symptom domains of ADHD possibly being influenced by different aetiological factors, it would make sense to look at the effects of the simplex/multiplex and shared/non-shared distinctions separately for the hyperactivity-impulsivity and inattention symptom dimensions. Furthermore, no such studies have been conducted to date.

Taking the above discussion into account, further evidence for the usefulness of the simplex/multiplex distinction in ADHD can be gathered by first determining the shared or non-shared nature of pregnancy and delivery complications, and then looking at the differential influence of these shared and non-shared risk factors on the disorder in multiplex versus simplex families. Should the simplex/multiplex distinction be proven meaningful in ADHD, non-shared environmental factors would be expected to play a greater role in simplex ADHD compared to multiplex ADHD. Alternatively, shared environmental factors would be expected to play a greater role in multiplex ADHD compared to simplex ADHD (Oerlemans, Burmanje, Franke, Buitelaar, Hartman, & Rommelse, 2016). Should the converse be true and the simplex/multiplex distinction not be useful in ADHD, non-shared and shared environmental factors should be shown to impact ADHD in simplex and multiplex families to an equal extent.

The aim of the current study was two-fold. Firstly, the shared or non-shared nature of pregnancy and delivery complications were determined. Secondly, the hypothesis was tested that non-shared pregnancy and delivery complications play a greater role in influencing the severity of ADHD symptoms in simplex families than in multiplex families. Conversely, pregnancy and delivery complications that are shared in nature play a greater role in the severity of symptoms in multiplex families compared to simplex families. Proving this hypothesis will provide further evidence for the usefulness of the simplex/multiplex distinction in the search for the causes of the disorder, possibly providing an avenue for reducing conflicting findings in the search for both molecular and environmental aetiological factors.
8.2 Methods

8.2.1 Participants

The sample consisted of a South African community sample of 171 siblings from 83 nuclear families. Only children between the ages of 5 and 18 years were included in the sample. However, to determine the simplex/multiplex nature of the disorder for each child, as well as to determine the shared or non-shared nature of pregnancy and delivery complications, data on ADHD diagnoses and pregnancy and delivery complications for all siblings 4 years and older were gathered. To be included in the sample, each nuclear family had to have at least one child diagnosed with ADHD. Children were classified as having ADHD if their parents reported that they had been diagnosed with the disorder by a healthcare professional. Children who were not diagnosed by a healthcare professional with the disorder according to parent-report, were seen as not having ADHD. This methodology has been employed in previous studies on ADHD (e.g. LeFever, Villers, & Morrow, 2002; Braun, Kahn, Froehlich, Auinger, & Lanphear, 2006) and especially due to the high costs and time-consuming nature of having each participant in the sample directly diagnosed by a healthcare professional. All singletons and adopted children were excluded from the analysis. Due to the exploratory nature of the study, no further exclusion criteria were applied. The final sample consisted of 108 children diagnosed with ADHD, and 63 children without the disorder.

8.2.2 Procedure

Parents who had at least one child diagnosed with ADHD were recruited through social media pages via the Attention Deficit and Hyperactivity Support Group of Southern Africa (ADHASA), as well as through medical professionals working with children diagnosed with ADHD in the Bloemfontein area. Parents were provided with an information leaflet to read (Appendix A1), as well as with an informed consent form to sign (Appendix A2). After providing consent to participate in the study, they were asked to complete the following measuring instruments:

(a) The SNAP-IV 26-item Teacher and Parent Rating Scale (Swanson, Kraemer, Hinshaw, Arnold, Conners, Abikoff, et al., 2001) (parent version) (Appendix B2). This rating scale is based on the DSM-IV criteria for ADHD in children, covering both symptoms
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of inattention and hyperactivity-impulsivity. Although the DSM-5 (APA, 2013) had recently been published, the symptoms listed for ADHD in this version of the DSM do not differ from the symptoms listed in the DSM-IV (APA, 1994), and therefore the SNAP-IV was seen as an appropriate rating scale to use in this study. A total score for ADHD symptom severity is derived from summing all items. The scale also includes items for measuring symptoms of Oppositional Defiant Disorder (ODD), but these items were not analysed in this chapter. The scale consists of 18 items measuring ADHD symptom severity, all measured on a four-point Likert scale ranging from zero (Not at all) to three (Very much).

(b) A self-compiled questionnaire gathering biographical information such as gender and age, as well as a question related to whether the child has been diagnosed with ADHD by a healthcare provider. In addition, parents were asked whether they experienced any complications during their pregnancy with each child, as well as whether there were any complications during the birth of each child (Appendix B1).

8.2.3 Statistical analysis

Data were cleaned prior to analysis and descriptive statistics calculated for all demographic and categorical variables. Mean imputation was used to replace missing values in the SNAP-IV scale (Siddiqui, 2015). Participants had to have answered at least seven out of the nine questions (83% of the 9 items - rounded) for both the inattention and hyperactivity-impulsivity symptom domains for means to be imputed. Any participants who answered fewer than seven out of nine questions for any one of the subscales were excluded from the inferential statistical analysis. To ensure the reliability of the measuring instruments, internal consistency reliability was calculated for both the inattention and hyperactivity-impulsivity subscales of the SNAP-IV scale.

Separate analyses were run where symptoms of inattention and symptoms of hyperactivity-impulsivity were entered as dependent variables respectively. The clustering of siblings within family units violated the assumption of independence of observations needed for standard multiple regression analysis (Tabachnick, 2006). However, generalised estimating equations modelling adjusts for correlations amongst siblings and has been proven effective in analysing family data (Homish, Edwards, Eiden, & Leonard, 2010;
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Hultman, Sandin, Levine, Lichtenstein, & Reichenberg, 2011). This technique is also robust as it requires no assumptions about data distribution (Hultman, Sandin, Levine, Lichtenstein, & Reichenberg, 2011). Therefore, this analysis technique was chosen for the current study. Robust standard errors were selected for all analyses, and to correct for possible dependence between siblings, family number was used as a repeated measure.

A variable was created representing simplex/multiplex ADHD through assigning a value of zero (Simplex) to all children from nuclear families where only one child was diagnosed with the disorder, and a value of one (Multiplex) to all children from families where more than one child was diagnosed with ADHD. A second variable was created where pregnancy and delivery complications were grouped into shared complications, non-shared complications, and no complications. If the same pregnancy or delivery complication was present in a child and one or more of his or her siblings, that child was seen to have had a shared complication. Conversely, if a complication was present only for the child in question, but not for his or her siblings, that child was seen to have had a non-shared complication. Children with no pregnancy or delivery complications were noted as such.

To determine whether the impact of the shared and non-shared pregnancy and delivery complications on ADHD symptom severity depended on whether a child was from a multiplex or simplex ADHD family, interaction effects between pregnancy and/or delivery complications (partitioned into shared-, non-shared, and no complications) and the simplex/multiplex variable were created for entry into the regression models. However, to ensure that the simplest models were chosen for testing the interaction effects, main effects models were first run for the effects of pregnancy and delivery complications on both inattention and hyperactivity-impulsivity scores. Only variables that were shown to have significant main effects on inattention and hyperactivity-impulsivity symptom severity were subsequently carried over to the full model which included the interaction effects represented by the product terms.

The significance level for all tests was set at an alpha value of 0.05 and all tests were two-sided (see Appendix E5 for extracts of the statistical analysis).
8.3 Results

8.3.1 Demographic characteristics

The average age of the sample was 10 years, with 110 (64.3%) males and 61 (35.7%) females (Table 8.1).

Table 8.1. Demographic characteristics of the sample.

<table>
<thead>
<tr>
<th>Age (Mean ± SD)</th>
<th>10 ± 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male (Count(%))</td>
<td>110 (64.3%)</td>
</tr>
<tr>
<td>Female (Count(%))</td>
<td>61 (35.7%)</td>
</tr>
</tbody>
</table>

8.3.2 Reliability of measuring instruments

The SNAP-IV rating scale subscales showed high internal consistency reliability in the current study, with Cronbach’s alpha coefficients of 0.96 for the inattention subscale and 0.94 for the hyperactivity-impulsivity subscale.

8.3.3 Pregnancy and delivery complications experienced

Pregnancy and delivery complications experienced by the participating mothers are listed in Table 8.2. The most frequently reported pregnancy complications were pre-eclampsia (7.0%), placental problems (2.9%), and infections during pregnancy (2.9%). Operative vaginal delivery (4.1%) and nuchal cord (4.1%) were the most frequently reported delivery complications.

8.3.4 Shared or non-shared nature of pregnancy and delivery complications

The pregnancy complications experienced in the current sample were both shared and non-shared in nature. There were 23 children whose mothers experienced similar complications during pregnancy with the child in question as with one or more of his or her siblings. In addition, 24 children’s mothers experienced pregnancy complications unique to that child. Finally, 124 children were from mothers who experienced no complications during their pregnancy with the child.
In the current sample, all children whose parents indicated that there were complications during the birth of the child only indicated the particular complication for one of their children. Thus, all delivery complications in this sample were found to be non-shared in nature. There were 38 children born with non-shared delivery complications and 133 children born with no delivery complications (Table 8.3).

### 8.3.5 Generalised estimating equations models results

**Main effects model 1: Pregnancy and delivery complications as predictors of inattention symptom severity.** As previously discussed, a main effects model was first run via generalised estimating equations to determine whether pregnancy complications and delivery complications, partitioned into shared and non-shared environmental factors, played a significant role in inattention symptom severity within the full sample.

**Table 8.2. Pregnancy and delivery complications.**

<table>
<thead>
<tr>
<th>Pregnancy complications</th>
<th>Frequency</th>
<th>Percent</th>
<th>Delivery complications</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No complications</td>
<td>123</td>
<td>71.9</td>
<td>No complications</td>
<td>133</td>
<td>77.8</td>
</tr>
<tr>
<td>Back problems</td>
<td>1</td>
<td>0.6</td>
<td>Breathing difficulties</td>
<td>4</td>
<td>2.3</td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>3</td>
<td>1.8</td>
<td>Breech baby</td>
<td>4</td>
<td>2.3</td>
</tr>
<tr>
<td>Coughing</td>
<td>1</td>
<td>0.6</td>
<td>Collapsed lung</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Deep vein thrombosis</td>
<td>1</td>
<td>0.6</td>
<td>Emergency caesarean – reason not specified</td>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td>Fetal growth restriction</td>
<td>1</td>
<td>0.6</td>
<td>Emergency caesarean - low heart rate</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Hyperemesis</td>
<td>4</td>
<td>2.3</td>
<td>Emergency caesarean - water broke due to infection</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Infections</td>
<td>5</td>
<td>2.9</td>
<td>Failure to progress in labour</td>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td>Placental problems</td>
<td>5</td>
<td>2.9</td>
<td>Fetal distress / Emergency caesarean</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>12</td>
<td>7.0</td>
<td>Fetal shock</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Pregnancy diabetes</td>
<td>4</td>
<td>2.3</td>
<td>Low heart rate</td>
<td>1</td>
<td>0.6</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Condition</th>
<th>Frequency</th>
<th>Percent</th>
<th>Condition</th>
<th>Frequency</th>
<th>Percent</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature labour</td>
<td>4</td>
<td>2.3</td>
<td>Neonatal pneumonia</td>
<td>1</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizure</td>
<td>1</td>
<td>0.6</td>
<td>Operative vaginal delivery</td>
<td>7</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>2</td>
<td>1.2</td>
<td>Placental abruption</td>
<td>1</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threatened miscarriage</td>
<td>3</td>
<td>1.8</td>
<td>Precipitous labour</td>
<td>1</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not specified</td>
<td>1</td>
<td>0.6</td>
<td>Nuchal cord</td>
<td>7</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not specified</td>
<td>1</td>
<td>0.6</td>
<td>Not specified</td>
<td>1</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8.3. Children born with shared and non-shared pregnancy and delivery complications.

<table>
<thead>
<tr>
<th></th>
<th>Shared complication</th>
<th>Non-shared complication</th>
<th>No complications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percent</td>
<td>Frequency</td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>23</td>
<td>13.5%</td>
<td>24</td>
</tr>
<tr>
<td>Delivery complications</td>
<td>0</td>
<td>0%</td>
<td>38</td>
</tr>
</tbody>
</table>

There was a significant main effect for complications occurring during the birth of the child, $\chi^2 (1) = 11.067; p = 0.001$ (Table 8.4). Recall that these complications were only non-shared in nature. Complications during pregnancy (shared or non-shared), however, had no significant effect on the severity of inattention symptoms, $\chi^2 (2) = 0.523; p = 0.770$. Children with delivery complications had a mean score for inattention symptom severity of 18.47 (Table 8.5). Children without delivery complications had a mean score of 13.54. Thus, children subjected to delivery complications had significantly more severe inattention symptoms than children with no delivery complications.
Table 8.4. Results of the main effects model for the effect of pregnancy and delivery complications on severity of inattention symptoms.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wald Chi-Square</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>412.814</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>0.523</td>
<td>2</td>
<td>0.770</td>
</tr>
<tr>
<td>Delivery complications</td>
<td>11.067</td>
<td>1</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Note. * p < 0.05; df = degrees of freedom; Sig. = p value

Table 8.5. Inattention symptom severity scores for children with and without delivery complications.

<table>
<thead>
<tr>
<th>Inattention symptom severity</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery complications (non-shared)</td>
<td>18.47</td>
<td>7.62</td>
</tr>
<tr>
<td>No delivery complications</td>
<td>13.54</td>
<td>9.43</td>
</tr>
</tbody>
</table>

Since only delivery complications had a significant effect on inattention severity symptoms in the current sample, pregnancy complications as a variable was excluded from further analysis on inattention symptoms.

Interaction effects model 1: Testing whether the effect of delivery complications on inattention symptom severity is dependent on the simplex/multiplex distinction. After finding a significant main effect for the non-shared delivery complications on inattention symptom severity in the full sample, a model was run testing whether the effects of delivery complications on inattention symptom severity were different for children from multiplex versus simplex ADHD families (Table 8.6).
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There was a significant interaction effect between whether a child was born with complications (non-shared) and whether a child is from a multiplex or simplex ADHD family, on ADHD symptom severity (Table 8.6). In other words, the effects of delivery complications (non-shared) on inattention symptom severity was different depending on whether a child came from a family where only one child was diagnosed with ADHD, or a family with more than one child diagnosed with the disorder. To glean the nature of this interaction effect, two separate models were run for the effects of delivery complications (non-shared) on inattention symptom severity for children from multiplex and simplex ADHD families respectively (simple main effects model) (Table 8.7). As is customary in the presence of a significant interaction effect (Fox, 2015), the main effects of simplex/multiplex ADHD and delivery complications (non-shared) were not explored further.

Table 8.6. Results of the model including an interaction effect between delivery complications and simplex/multiplex ADHD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wald Chi-Square</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>602.570</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Delivery complications (non-shared)</td>
<td>8.325</td>
<td>1</td>
<td>0.004*</td>
</tr>
<tr>
<td>Simplex/multiplex ADHD</td>
<td>13.066</td>
<td>1</td>
<td>0.000*</td>
</tr>
<tr>
<td>Delivery complications X Simplex/multiplex ADHD</td>
<td>4.909</td>
<td>1</td>
<td>0.027*</td>
</tr>
</tbody>
</table>

Note. * p < 0.05; df = degrees of freedom; Sig. = p value
Table 8.7. Results of the simple main effects model for the effects of delivery complications on inattention symptom severity within simplex and multiplex families.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE_B</th>
<th>Wald Chi-Square</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children from simplex ADHD families</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>10.967</td>
<td>0.663</td>
<td>273.648</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Delivery Complications</td>
<td>6.879</td>
<td>1.740</td>
<td>15.640</td>
<td>1</td>
<td>0.000*</td>
</tr>
<tr>
<td>Children from multiplex ADHD families</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>18.930</td>
<td>1.166</td>
<td>263.791</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Delivery Complications</td>
<td>0.903</td>
<td>2.061</td>
<td>0.192</td>
<td>1</td>
<td>0.661</td>
</tr>
</tbody>
</table>

Note. * p < 0.05; B = unstandardized regression coefficient; SE\_B = standard error of the coefficient; df = degrees of freedom; Sig. = p value

For children from multiplex ADHD families, the non-shared delivery complications had no significant effect on the severity of inattention symptoms, \( \chi^2 (1) = 0.098; p = 0.755 \). In contrast, for children from simplex ADHD families, the non-shared delivery complications did have a significant effect on the severity of inattention symptoms, \( \chi^2 (1) = 15.640; p = 0.000 \). Children from simplex ADHD families with non-shared delivery complications had a mean inattention symptom severity score of 17.85, whereas children with no delivery complications had a mean score of 10.97 (Table 8.8). However, for children from multiplex ADHD families, the difference in inattention symptom severity scores of those exposed to the non-shared delivery complications versus those not exposed were slight. Children with delivery complications (non-shared) had a mean inattention symptom severity score of 19.83 compared to 18.93 for children without delivery complications (non-shared). Thus, children subjected to the non-shared delivery complications had significantly more severe inattention symptoms than children with no delivery complications, but only if they were from families with only one child diagnosed with ADHD.
Table 8.8. The effect of delivery complications on mean inattention symptom severity scores for children from simplex and multiplex ADHD families.

<table>
<thead>
<tr>
<th></th>
<th>Inattention symptom severity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>Children from simplex ADHD</td>
<td>Delivery complications</td>
<td>17.85</td>
<td>7.98</td>
</tr>
<tr>
<td>families</td>
<td>No delivery complications</td>
<td>10.97</td>
<td>9.18</td>
</tr>
<tr>
<td>Children from multiplex</td>
<td>Delivery complications</td>
<td>19.83</td>
<td>6.89</td>
</tr>
<tr>
<td>ADHD families</td>
<td>No delivery complications</td>
<td>18.93</td>
<td>7.53</td>
</tr>
</tbody>
</table>

Main effects model 2: Pregnancy and delivery complications as predictors of hyperactivity-impulsivity symptom severity. A second main effects model was run, also via generalised estimating equations modelling, to determine whether pregnancy complications and delivery complications, partitioned into shared and non-shared environmental factors, played a significant role in hyperactivity-impulsivity symptom severity within the sample as a whole (Table 8.9).

Table 8.9. Results of the main effects model for the effect of pregnancy and delivery complications on severity of hyperactivity-impulsivity symptoms.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wald Chi-Square</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>276.691</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>0.377</td>
<td>2</td>
<td>0.828</td>
</tr>
<tr>
<td>Delivery complications</td>
<td>5.936</td>
<td>1</td>
<td>0.015*</td>
</tr>
</tbody>
</table>

Note. * p < 0.05; df = degrees of freedom; Sig. = p value

As was the case for inattention symptom severity, complications during the birth of the child again had a significant impact on the severity of hyperactivity-impulsivity symptoms, $\chi^2 (1) = 6.045; p = 0.014$, but no significant effect could be detected for pregnancy complications
\( \chi^2 (1) = 0.397; p = 0.529 \) (Table 8.9). Recall that all delivery complications were non-shared in nature. Children with non-shared delivery complications had a mean score for hyperactivity-impulsivity symptom severity of 15.03 compared to a mean score of 11.41 for children with no delivery complications (Table 8.10). Thus, as was the case with inattention symptom severity, children subjected to the non-shared delivery complications had more severe hyperactivity-impulsivity symptoms than children with no delivery complications.

**Table 8.10. Hyperactivity-impulsivity symptom severity scores for children with and without delivery complications.**

<table>
<thead>
<tr>
<th>Hyperactivity-impulsivity symptom severity</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery complications (non-shared)</td>
<td>15.03</td>
<td>8.26</td>
</tr>
<tr>
<td>No delivery complications</td>
<td>11.41</td>
<td>9.10</td>
</tr>
</tbody>
</table>

Since only delivery complications had a significant effect on hyperactivity-impulsivity symptom severity in the full sample, pregnancy complications as a variable was excluded from further analysis of hyperactivity-impulsivity.

**Interaction effects model 2: Testing whether the effect of delivery complications on hyperactivity-impulsivity symptom severity is dependent on the simplex/multiplex distinction.** After finding a significant main effect for the non-shared delivery complications on hyperactivity-impulsivity symptom severity in the full sample, a model was run testing whether the effects of delivery complications (non-shared) on hyperactivity-impulsivity symptom severity were different for children from multiplex versus simplex ADHD families (Table 8.11).
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There was no significant interaction effect between delivery complications (non-shared) and simplex/multiplex ADHD on hyperactivity-impulsivity symptom severity (Table 8.11). There were, however, significant main effects for both delivery complications (non-shared), $\chi^2 (1) = 5.277; p = 0.022$ and simplex/multiplex ADHD, $\chi^2 (1) = 6.568; p = 0.010$. The main effect of delivery complications on hyperactivity-impulsivity symptoms has already been discussed.

Children from families with more than one child diagnosed with ADHD (multiplex families) had a higher mean hyperactivity-impulsivity score (Mean = 15.58) than children from families with only one child diagnosed with the disorder (simplex families) (Mean = 10.61) (Table 8.12).

Table 8.11. Results of the model including an interaction effect between delivery complications and simplex/multiplex ADHD on hyperactivity-impulsivity symptom severity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wald Chi-Square</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>279.192</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Delivery Complications</td>
<td>5.188</td>
<td>1</td>
<td>0.023*</td>
</tr>
<tr>
<td>Simplex/multiplex ADHD</td>
<td>6.411</td>
<td>1</td>
<td>0.011*</td>
</tr>
<tr>
<td>Delivery Complications X Simplex/multiplex ADHD</td>
<td>0.945</td>
<td>1</td>
<td>0.331</td>
</tr>
</tbody>
</table>

Note. * $p < 0.05$; df = degrees of freedom; Sig. = $p$ value

Table 8.12. Hyperactivity-impulsivity symptom severity scores for children from simplex and multiplex ADHD families.

<table>
<thead>
<tr>
<th>Hyperactivity-impulsivity symptom severity</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children from simplex ADHD families</td>
<td>10.61</td>
<td>8.77</td>
</tr>
<tr>
<td>Children from multiplex ADHD families</td>
<td>15.58</td>
<td>8.69</td>
</tr>
</tbody>
</table>
8.4 Discussion

Cronbach’s alpha coefficients for the subscales of the SNAP-IV scale were high. This result is in line with results from previous studies, with Bussing, Fernandez, Harwood, Wei, Garvan, Eyberg, et al. (2008) reporting Cronbach’s alpha coefficients of 0.90 for the inattention subscale and 0.79 for the hyperactivity-impulsivity subscale in a sample of elementary school students in the United States of America. The SNAP-IV scale was thus a reliable instrument for the measurement of inattention and hyperactivity-impulsivity in the current sample. Due to the non-probability sampling method used, the current sample was not representative of the South African population. This is common in behavioural genetic studies published in peer-reviewed journals (Agudelo, Gálvez, Fonseca, Mateus, Taler-Gutiérrez, & Velez-Van-Meerebecke, 2015; Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Van Dyk, Springer, Kidd, Steyn, Solomons, & Van Toorn, 2014), but nonetheless precludes the generalization of the findings to the South African population at large.

All delivery complications in the current study were found to be non-shared in nature. However, in line with the hypothesis set by Amor, Grizenko, Schwartz, Lageix, Baron, Ter-Stepanian, et al. (2005), pregnancy complications were found to be both shared and non-shared in nature. Thus, some pregnancy complications were present for only one child in a family, whereas other complications occurred in more than one sibling within a nuclear family.

Testing the main effects of pregnancy and delivery complications, partitioned into shared-, non-shared and no complications, on inattention and hyperactivity-impulsivity symptom severity showed that there was a significant main effect for the non-shared delivery complications, but not for pregnancy complications. The non-significant result for pregnancy complications means that there was no significant difference in either hyperactivity-impulsivity or inattention symptom severity between children whose mothers experienced shared pregnancy complications, non-shared pregnancy complications, or no pregnancy complications. The non-significant result for pregnancy complications is in line with previous research showing no significant effect for factors such as operative procedures, gestational diabetes, pre-eclampsia, placental abnormalities, infections, chronic maternal
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disease, and bleeding (St Sauver, Barbaresi, Katusic, Colligan, Weaver, & Jacobsen, 2004; Wagner, Schmidt, Lemery-Chalfant, Leavitt, & Goldsmith, 2009), all factors that were present in this sample as well. In addition, the current results are in contrast to that reported by Buschgens, Swinkels, Van Aken, Ormel, Verhulst, and Buitelaar (2009) and Ketzer, Gallois, Martinez, Rohde, and Schmitz (2012). It should however be noted that in both latter studies, the researchers created a combined index for pregnancy and delivery complications. It is therefore possible that it was actually the delivery complications in this index that were responsible for the significant main effects found, and not the pregnancy complications. Moreover, the study by Buschgens, Swinkels, Van Aken, Ormel, Verhulst, and Buitelaar (2009) included social or psychological problems during pregnancy in their index of pregnancy complications. These factors were not assessed in the current study. Nonetheless, previous research has shown that social or psychological problems during pregnancy, maternal stress in particular, play a role in ADHD symptomology (Grizenko, Fortier, Gaudreau-Simard, Jolicoeur, & Joober, 2015).

The significant main effect for delivery complications (only non-shared) on inattention and hyperactivity-impulsivity symptom severity showed that children who did experience non-shared complications during birth had higher inattention and hyperactivity-impulsivity scores than children with no delivery complications. The finding regarding inattention symptom severity was congruent with the results reported by Buschgens, Swinkels, Van Aken, Ormel, Verhulst, and Buitelaar (2009) and Ketzer, Gallois, Martinez, Rohde, and Schmitz (2012), who, like this study, reported on complications such as breech presentation, caesarean section, lack of oxygen, blood transfusion, and jaundice. Although Ketzer, Gallois, Martinez, Rohde, and Schmitz (2012) did not investigate the hyperactivity-impulsivity symptom domain, the significant main effect found on hyperactivity-impulsivity symptom severity contrasts with the finding by Buschgens, Swinkels, Van Aken, Ormel, Verhulst, and Buitelaar (2009). Latter researchers found a significant main effect for pregnancy and delivery complications on inattention, but not on hyperactivity-impulsivity. The hyperactivity-impulsivity results are also in contrast to that reported by St Sauver, Barbaresi, Katusic, Colligan, Weaver, and Jacobsen (2004) and Wagner, Schmidt, Lemery-Chalfant, Leavitt, and Goldsmith (2009). However, the latter two studies did not consider
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hyperactivity-impulsivity and inattention separately, but rather focused on ADHD symptoms and diagnosis as a single disorder.

The significant main effects found for both hyperactivity-impulsivity and inattention prompted further investigation into whether the effect of delivery complications (non-shared) on symptom severity is similar in simplex and multiplex ADHD families. For this subsequent analysis, results for the inattention and hyperactivity-impulsivity symptom domains diverged. A significant interaction effect between the non-shared delivery complications and simplex/multiplex ADHD on inattention scores were found, but no such effect could be detected for hyperactivity-impulsivity. Thus, for inattention, the effect of non-shared delivery complications depended on the simplex/multiplex family distinction, but for hyperactivity-impulsivity the effect was similar for both simplex and multiplex ADHD families. The difference in the current findings for the two symptom dimensions might explain the results found by Oerlemans, Burmanje, Franke, Buitelaar, Hartman, and Rommelse (2016) where the simplex/multiplex distinction added no additional insight into the effects of pre- and perinatal environmental insults on ADHD. Like St Sauver, Barbaresi, Katusic, Colligan, Weaver, and Jacobsen (2004) and Wagner, Schmidt, Lemery-Chalfant, Leavitt, and Goldsmith (2009), Oerlemans, Burmanje, Franke, Buitelaar, Hartman, and Rommelse (2016) also did not look at hyperactivity-impulsivity and inattention separately. This indicates that partitioning ADHD into its respective symptom dimensions may aid in the search for aetiological factors.

Although a significant interaction effect between simplex/multiplex ADHD and non-shared delivery complications was not found for hyperactivity-impulsivity, there was a significant main effect for the simplex/multiplex distinction on hyperactivity-impulsivity symptom severity. Children from multiplex families had significantly higher mean hyperactivity-impulsivity symptom severity scores than children from simplex families. This finding is in line with the hypotheses set by Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al. (2014) and Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, and Rommelse (2015) where multiplex ADHD was postulated to be influenced by factors shared between family members, resulting in all family members showing symptoms. Simplex ADHD was however postulated to be influenced by factors specific to the individual, with no influence
on the unaffected family members. This would result in inflated symptom scores for unaffected individuals from multiplex families, but not simplex families. These inflated symptom scores would in turn elevate the mean symptom scores of individuals from multiplex families compared to individuals from simplex families and may have resulted in the significantly greater mean hyperactivity-impulsivity scores for multiplex families witnessed here. Although further study is required, this finding provides evidence for the simplex/multiplex distinction being valid and applicable to the hyperactivity-impulsivity symptom dimension.

Further investigation into the significant interaction effect between simplex/multiplex ADHD and non-shared delivery complications on inattention symptom severity showed that the effect of non-shared delivery complications on inattention symptom severity was only applicable to children from a nuclear family where only one child has been diagnosed with ADHD (simplex ADHD). Thus, children born with non-shared delivery complications had more severe inattention symptoms, but only if they were from a simplex ADHD family. In families with more than one child diagnosed with ADHD (multiplex ADHD), non-shared delivery complications had no significant effect on inattention symptom severity. This finding is in line with the hypothesis that, should the simplex/multiplex distinction be valid for ADHD, non-shared environmental factors would influence symptom severity in simplex ADHD, but not in complex ADHD (Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar, et al., 2014; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015).

Although the influence of shared genetic factors, shared environmental factors, and non-shared genetic factors could not be tested in this study, the above results for both the inattention and hyperactivity-impulsivity symptom dimensions are in line with findings that would be expected should simple and multiplex ADHD represent two aetiologically distinct forms of the disorder, as proposed by various authors (Freitag, 2007; Oerlemans, Hartman, De Bruijn, Steijn, Franke, Buitelaar et al., 2014; Oerlemans, Hartman, De Bruijn, Franke, Buitelaar, & Rommelse, 2015, Oerlemans, Burmanje, Franke, Buitelaar, Hartman, & Rommelse, 2016; Sebat, Lakshmi, Malhotra, Troge, Lese-Martin, Walsh, et al., 2007; Sullivan, Daly, & O’Donovan, 2012).
8.5 Conclusion

In conclusion, pregnancy complications, which were found to be both shared and non-shared in nature, were not associated with either inattention or hyperactivity-impulsivity symptom severity. Delivery complications, which were found to be only non-shared in nature, do seem to be significantly associated with both the inattention and hyperactivity-impulsivity symptom dimensions of ADHD. Children exposed to non-shared delivery complications display more severe inattention and hyperactivity-impulsivity symptoms than children not exposed to delivery complications.

The influence of delivery complications on hyperactivity-impulsivity was not dependent on whether a child was from a multiplex or simplex ADHD family. That said, individuals from multiplex families showed significantly higher mean hyperactivity-impulsivity symptoms severity scores than individuals from simplex families. This result would be expected if simplex ADHD is indeed only influenced by factors unique to the individual, with no influence on other family members, whereas multiplex ADHD is influenced by factors shared between family members, affecting everyone in the family, and resulting in higher mean symptom scores.

In contrast to the hyperactivity-impulsivity symptom dimension, the influence of delivery complications on inattention seemed to be limited only to children from simplex ADHD families. The differential effect of non-shared delivery complications on inattention symptom severity in simplex versus multiplex ADHD families supports the hypothesis that simplex and multiplex ADHD represent two aetiologically distinct forms of the disorder, with simplex ADHD mainly influenced by non-shared genetic and environmental factors, and multiplex ADHD influenced only by shared genetic and environmental factors. Although further study is needed, the findings from this study do provide evidence for the usefulness of the simplex/multiplex distinction for both inattention and hyperactivity-impulsivity. Distinguishing between simplex and multiplex ADHD might provide an avenue for reducing conflicting findings in the search for both molecular and environmental aetiological factors.
8.6 Limitations of the study

Several limitations to this study are important to mention. Firstly, the exploratory nature of the study resulted in the researchers relaxing the exclusion criteria for the sample. Future studies that are less explorative in nature should consider applying more stringent exclusion criteria before aiming to replicate the results. The study also only used the ADHD diagnostic status of children in the nuclear families to assign families to the multiplex or simplex ADHD categories. It might be worthwhile in future research to look at parental ADHD status as well prior to distinguishing families as simplex or multiplex for the disorder. In addition, the classification of children as being diagnosed with ADHD or not was made according to parent-report of diagnosis by a healthcare professional. It would have been preferable to have all participants directly assessed for the disorder by healthcare providers, but this fell outside the budget and time available for this project. Lending some credence to the methodology employed here is that it has been used before in published research as noted, and also that a study by Visser, Danielson, Bitsko, Perou, and Blumberg (2013) found no statistically significant differences in the prevalence rate of ADHD diagnosis of children as reported by parents and that gleaned directly from the medical records of healthcare providers. Furthermore, this study used retrospective recall of parents for the assessment of pregnancy and delivery complications. Research have shown that retrospective recall related to pre- and perinatal complications may be problematic, depending on the characteristics being recalled. Nevertheless, for some characteristics, recall was accurate even 22 or more years after the event (Buka, Goldstein, Spartos, & Tsuang, 2004; Sou, Chen, Hsieh, & Jeng, 2006).

A further limitation in this study was that, due to the non-probability sampling strategy employed, the sample was not representative of the South African population. Although this is common in published behavioural genetic studies, as noted, this precludes the generalization of findings to the South African population at large. The statistical techniques and family study methodology employed in this study can however serve as a blueprint for future studies conducted on the simplex/multiplex nature of ADHD in South Africa. Finally, although delivery complications being only non-shared in nature added power and thus strength to the statistical analysis, it also resulted in the limitation that the effect of shared
environmental factors significantly impacting ADHD could not be tested. Larger sample sizes in future studies may prove useful in providing samples with both shared and non-shared delivery complications present, making it possible to test the differential effect of shared and non-shared delivery complications in multiplex versus simplex ADHD families.
Chapter 9

The influence of the interaction between a polymorphism in the dopamine transporter gene and pregnancy and/or delivery complications on the severity of ADHD symptoms in a South African sample

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This chapter is the first chapter in the thesis to explore the molecular genetic architecture of Attention-Deficit/Hyperactivity Disorder (ADHD). A possible reason for the mixed findings is that genetic factors interact with factors in the environment to influence the disorder. In this chapter, a possible interaction effect between a polymorphism in the dopamine transporter gene and factors in the maternal and prenatal environment is explored. The research conducted, the statistical analysis, and the writing of this chapter was done by Nadia Fouché.

Acknowledgements: Financial support by the National Research Foundation, South Africa, is greatly acknowledged.
Abstract

Numerous studies have been conducted on the possible influence of a variable number of tandem repeats polymorphism in the 3’ untranslated region of the dopamine transporter gene (DAT1 3’ uVNTR) on ADHD. The results of these studies have been conflicting, leading researchers to suggest a possible interaction effect between an environmental factor and this polymorphism. The current study tested the hypothesis that an interaction exists between pregnancy and/or delivery complications and the DAT1 3’ uVNTR on ADHD symptoms of inattention and hyperactivity-impulsivity. A South African community sample of 51 children between the ages of 5 and 18 years, enriched with diagnoses of ADHD, was genotyped for the DAT1 3’ uVNTR. Information on pregnancy and/or birth complications were gathered from the parents. Inattention and hyperactivity-impulsivity symptom severity scores were gathered for the sample by means of the parent version of the SNAP-IV rating scale. Two-way analysis of variance revealed a significant interaction between pregnancy and/or delivery complications and DAT1 3’ uVNTR genotype. Pregnancy and/or delivery complications resulted in a significant increase in both inattention and hyperactivity-impulsivity scores, but only for children homozygous for the 10-repeat allele of the DAT1 3’ uVNTR. This result points to an indirect, rather than a direct effect of the DAT1 3’ uVNTR on ADHD symptom severity, providing a possible mechanism explaining the conflicting findings.

Keywords  Dopamine transporter, Hyperactivity-impulsivity, Inattention, Pregnancy and delivery complications, Two-way analysis of variance, Variable number of tandem repeats polymorphism
9.1 Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a highly prevalent, childhood onset, externalising disorder, characterised by symptoms of inattention and/or hyperactivity-impulsivity (American Psychiatric Association [APA], 2013; Polanczyk, De Lima, Horta, Biederman, & Rohde, 2007). The disorder has been shown to have far reaching negative ramifications, negatively impacting children’s peer-relations, academic achievements, and family life (Foley, 2011; Hoza, 2007; Hoza, Mrug, Gerdes, Hinshaw, Bukowski, Gold, et al., 2005; Loe, & Feldman, 2007; Mrug, Molina, Hoza, Gerdes, Hinshaw, Hechtman, et al., 2012; Pheula, Rohde, & Schmitz, 2011). In the newest version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), three distinct presentations of the disorder are specified, namely combined presentation, predominantly inattentive presentation, and predominantly hyperactive-impulsive presentation. Children with predominantly inattentive presentation mostly display symptoms of inattention. Likewise, children with predominantly hyperactive-impulsive presentation mostly display symptoms of hyperactivity-impulsivity. Children with combined presentation display symptoms of both inattention and hyperactivity-impulsivity (APA, 2013).

Research has proven that ADHD is a highly heritable disorder. A meta-analysis of twin and adoption studies conducted by Nikolas, and Burt (2010) found that genetic factors accounted for more than 70% of the variance in both inattention and hyperactivity-impulsivity symptom scores. Not surprisingly, numerous molecular genetic studies have been conducted in an attempt to find genes associated with the disorder (Li, Chang, Zhang, Gao, & Wang, 2014). One of the most frequently studied candidate gene polymorphisms in ADHD, that have also produced some of the most conflicting findings, is the variable number of tandem repeats (VNTR) polymorphism in the 3’ untranslated region (UTR) of the dopamine transporter gene (DAT1) (this polymorphism will henceforth be referred to as the DAT1 3’ uVNTR) (Faraone, & Mick, 2010; Gatt, Burton, Williams, & Schofield, 2015; Gizer, Ficks, & Waldman, 2009; Li, Chang, Zhang, Gao, & Wang, 2014).

Vandenbergh, Persico, Hawkins, Griffin, Li, Jabs, et al. (1992) mapped the DAT1 gene to the short arm of chromosome 5 (5p15). This gene has been shown to code for a protein known as the dopamine transporter (DAT) protein. This protein is responsible for the...
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reuptake of the neurotransmitter dopamine from the synaptic cleft of dopaminergic neurons, back into the presynaptic neuron, and thus terminates dopamine neurotransmission (Amara, & Kuhar, 1993; Ciliax, Drash, Staley, Haber, Mobley, Miller, et al., 1999; Giros, Mestikawy, Godinot, Zheng, Han, Yang-Feng, et al., 1992). The VNTR in the 3’ UTR of the DAT1 gene was first reported on by Vandenbergh, Persico, Hawkins, Griffin, Li, Jabs, et al. (1992). This polymorphism consists of a 40 base pair sequence repeated a variable number of times. The most common alleles consist of 10 repeats and nine repeats respectively (Agudelo, Gálvez, Fonseca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Doucette-Stamm, Blakely, Tian, Mockus, & Mao, 1995; Gizer, Ficks, & Waldman, 2009; Kang, Palmatier, & Kidd, 1999; Santovito, Cervella, Selvaggi, Caviglia, Burgarello, Sella, et al., 2008).

Numerous studies have been performed to determine whether the DAT1 3’ uVNTR affects DAT expression and DAT binding (for a review see Willeit, & Praschak-Rieder, 2010). Most in-vitro studies on the association between the DAT1 3’ uVNTR and DAT expression found higher DAT expression in individuals homozygous for the 10-repeat allele (Brookes, Neale, Sugden, Khan, Asherson, & D’Souza, 2007; Fuke, Suo, Takahashi, Koike, Sasagawa, & Ishiura., 2001; Mill, Asherson, Browes, D’Souza, & Craig, 2002; VanNess, Owens, & Kilts, 2005; Willeit & Praschak-Rieder, 2010). That said, in-vitro studies have also found the 9-repeat allele to be associated with greater DAT expression (Miller, & Madras, 2002), as well as no association between this polymorphism and DAT1 gene expression (Mill, Asherson, Craig, & D’Souza, 2005; Pinsonneault, Han, Burdick, Kataka, Bertolino, Malhotra, et al., 2011).

In contrast to the majority of in-vitro studies, several in-vivo studies reported either the opposite, with the presence of the 9-repeat allele being associated with increased availability/binding of the DAT protein (Faraone, Spencer, Madras, Zhang-James, & Biederman, 2014; Jacobsen, Staley, Zoghbi, Seibyl, Kosten, Innis, et al., 2000; Van de Giessen, De Win, Tanck, Van den Brink, Baas, & Booij, 2009; Van Dyck, Malison, Jacobsen, Seibyl, Staley, Laruelle, et al., 2005), or found no association between this polymorphism and DAT availability/binding (Krause, Dresel, Krause, Fougère, Zill, & Ackenheil, 2006; Lafuente, Bernardo, Mas, Crescenti, Aparici, Gassó, et al., 2007; Lynch, Mozley, Sokol, Maas, Balcer, & Siderowf, 2003; Martinez, Gelernter, Abi-Dargham, Van Dyck, Kegeles, Innis, et al., 2001). As
concluded in the review by Willeit, and Praschak-Rieder (2010), the effect of the DAT1 3’ uVNTR on DAT expression, availability, and binding is still not clear. However, it does seem that this polymorphism influences the expression, availability, and/or binding of DAT one way or the other. In turn, the availability of the DAT has been shown to have a crucial impact on the regulation of synaptic dopamine levels and dopamine transmission (Faraone, Spencer, Madras, Zhang-James, & Biederman, 2014; Fuke, Suo, Takahashi, Koike, Sasagawa, & Ishiura, 2001; Jaber, Jones, Giros, & Caron, 1997).

Evidence for the association of ADHD with dopamine function and the DAT mainly comes from studies on the workings of methylphenidate (MPH), seen by various researchers as the most effective pharmacological treatment for ADHD symptoms (Gizer, Ficks, & Waldman, 2009; Hanwella, Senanayake, & De Silva, 2011; Maia, Cortese, Caye, Deakin, Polanczyk, Polanczyk, et al., 2017; Subcommittee on Attention-Deficity/Hyperactivity Disorder, Steering committee on quality improvement and management, 2011; Volkow, Wang, Fowler, Gatley, Logan, Ding, et al., 1998). Studies using positron-emission tomography (PET) found that, at the therapeutic dose used for ADHD, MPH blocked up to 70% of brain dopaminergic transporters (Volkow, Wang, Fowler, Gatley, Logan, Ding, et al., 1998; Zimmer, 2017). Therefore, an increase in the extracellular concentration of dopamine in the human brain is observed, which is considered the main mechanism of methylphenidate’s therapeutic efficacy (Gizer, Ficks, & Waldman, 2009; Spencer, Devilbiss, & Berridge, 2015; Volkow, Wang, Fowler, Gatley, Logan, Ding, et al., 1998; Volkow, Wang, Fowler, Logan, Gerasimov, Maynard, et al., 2001).

Given the crucial role of the DAT as a binding site for MPH, along with the medication’s efficacy and the evidence for the DAT1 3’ uVNTR influencing the DAT, it is not surprising that so many candidate gene studies have been conducted on a possible role of this polymorphism in ADHD. As previously mentioned, results from these studies have been conflicting. The first study to find a significant association between the DAT1 3’ uVNTR and ADHD reported that the 10-repeat allele significantly increased risk for ADHD (Cook, Stein, Krasowski, Cox, Olkon, Kieffer, et al., 1995). However, in a subsequent meta-analysis conducted by Li, Sham, Owen, and He (2006), no significant association between the 10-repeat allele of the DAT1 3’ uVNTR and risk for ADHD could be found. In a later
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comprehensive meta-analysis by Gizer, Ficks, and Waldman (2009), results from 35 studies conducted between 1995 and 2009 were pooled and odds ratios calculated. The researchers reported an odds ratio of 1.12 for the 10-repeat allele of the DAT1 3’ uVNTR, indicating a modest but significant association between this allele and ADHD. That said, in the meta-analysis by Gizer, Ficks, and Waldman (2009), as well as in two subsequent reviews (Faraone, & Mick, 2010; Gatt, Burton, Williams, & Schofield, 2015), the conflicting effects found across studies for the association between the DAT1 3’ uVNTR and ADHD is highlighted. This high degree of heterogeneity led the researchers to allude to the possible influence of environmental factors interacting with this polymorphism to influence ADHD (Faraone, & Mick, 2010; Gizer, Ficks, & Waldman, 2009).

Environmental factors interacting with genes has proven an important tool for providing insight into the aetiology of psychiatric disorders (Halldorsdottir, & Binder, 2017). Gene-environment interactions (GXEs) are particularly of interest in neurodevelopmental disorders, given the plasticity of the young brain and its consequent vulnerability to environmental insults (Pietropaolo, Crusio, & Feldon, 2017). Environmental factors that have been shown to influence ADHD in particular relate to factors surrounding pregnancy and birth (Grizenko, Fortier, Zadorozny, Thakur, Schmitz, Duval, et al., 2012; Gustafsson, & Källén, 2011; Wiggs, Elmore, Nigg, & Nikolas, 2016; Zhu, Gan, Huang, Li, Qu, & Mu, 2016). Factors in the pre- and peri-natal environment that have been found to influence ADHD include perinatal hypoxic-ischemic conditions such as preeclampsia, Apgar score below seven at five minutes, breech or transverse presentation, foetal distress, foetal post-maturity, duration of labour, and prolapsed/nuchal cord (Banerjee, Middleton, & Faraone, 2007; Zhu, Gan, Huang, Li, Qu, & Mu, 2016). In addition, antepartum haemorrhage, young maternal age, maternal smoking and/or alcohol use during pregnancy, low birth weight, and preterm birth have all been found to be associated with ADHD (Banerjee, Middleton, & Faraone, 2007; Buschgens, Swinkels, Van Aken, Ormel, Verhulst, & Buitelaar, 2009; Gustafsson, & Källén, 2011; Knopik, Sparrow, Madden, Bucholz, Hudziak, Reich, et al., 2005; Wagner, Schmidt, Lemery-Chalfant, Leavitt, & Goldsmith, 2009).

Although numerous studies have been conducted on possible interaction effects between the DAT1 3’ uVNTR polymorphism and maternal smoking or alcohol use during
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pregnancy (Becker, El-Faddagh, Schmidt, Esser, & Laucht, 2008; Brookes, Mill, Guindalini, Curran, Xu, Knight, et al., 2006; Ficks, & Waldman, 2009; Kahn, Khoury, Nichols, & Lanphear, 2003; Neuman, Lobos, Reich, Henderson, Sun, & Todd, 2007; Nigg, Nikolas, & Burt, 2010; Schachar, 2014), surprisingly few studies have looked at possible interaction effects between this polymorphism and other pregnancy and/or delivery complications. Only two studies could be found in this regard, both testing for possible interaction effects between low birth weight and the DAT1 3’ uVNTR (Jackson, & Beaver, 2015; Langley, Turic, Rice, Holmans, Van den Bree, Craddock, et al., 2008). However, the results from the two studies were conflicting. Jackson, and Beaver (2015) reported a significant interaction effect between the presence of the 10-repeat allele of the DAT1 3’ uVNTR and low birth weight, with carriers of the 10-repeat allele who weighed less than 2.27 kg at birth being at increased risk of exhibiting ADHD symptoms. In contrast, Langley, Turic, Rice, Holmans, Van den Bree, Craddock, et al. (2008) found no significant interaction effect between the DAT1 3’ uVNTR and low birth weight on ADHD.

It is therefore clear that there is a paucity of studies on the possible interaction effects between the DAT1 3’ uVNTR and pre- and perinatal environmental factors, other than maternal alcohol use and smoking on ADHD symptom severity. The aim of this study was to fill this gap by examining possible interaction effects between DAT1 3’ uVNTR genotype and the presence of pre- and perinatal risk factors, excluding maternal substance use, on ADHD symptom dimensions of inattention and hyperactivity-impulsivity. This will be the first study of its kind to be conducted in a South African sample and can therefore serve as a basis for future research into gene-environment interactions influencing ADHD in South Africa specifically. The hypothesis for this study is that the influence of pregnancy and/or birth complications on ADHD inattention and hyperactivity-impulsivity symptom severity scores will depend on the DAT1 3’ uVNTR genotype. As was done in previous studies (Gizer, Ficks, & Waldman, 2009; Jackson, & Beaver, 2015), the 10-repeat allele of the DAT1 3’ uVNTR was considered the risk allele.
9.2 Methods
9.2.1 Participants

The sample consisted of a South African community sample of 51 children between the ages of 5 and 18 years, enriched with diagnoses of ADHD\(^2\). All adopted children and children falling outside the 5 to 18 year age range were excluded from the sample. Due to the exploratory nature of the study, no further exclusion criteria were applied. As was done in previous studies (e.g. LeFever, Villers, & Morrow, 2002; Braun, Kahn, Froehlich, Auinger, & Lanphear, 2006) classification of children as being diagnosed with ADHD or not was based on parent-report of ADHD diagnosis by a healthcare professional. Children who parents reported had received a diagnosis of ADHD by a healthcare professional were classified as having ADHD, whereas children who parents reported were not diagnosed with the disorder by a healthcare professional were seen as not having ADHD. For this subset of the sample, the ADHD diagnostic status of all children who were indicated by their parents as having been diagnosed with ADHD were directly confirmed by the diagnosing healthcare professional\(^3\).

9.2.2 Procedure

The sample was recruited through social media pages via the Attention Deficit and Hyperactivity Support Group of Southern Africa (ADHASA), as well as through medical professionals working with children diagnosed with ADHD in the Bloemfontein area, Free State Province of South Africa. Parents were provided with information leaflets for the study being conducted (Appendix A1) and for genetic research (Appendix A3). In addition, parents were provided with two informed consent forms to sign. One for consenting to complete questionnaires for their children (Appendix A2) and the other for consenting to allow their children to participate in genetics research (Appendix A4). After provision of consent to participate in the study, parents were asked to complete the following measuring instruments:

a) The SNAP-IV 26-item Teacher and Parent Rating Scale (Swanson, Kraemer, Hinshaw, Arnold, Conners, Abikoff, et al., 2001) (parent version) is based on the DSM-IV criteria

\(^2\) Numerous children in the sample were diagnosed with ADHD by a healthcare provider according to parent-report

\(^3\) Paediatrician: Prof Andre Venter; Clinical psychologist: Lida van Zyl
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for ADHD in children, covering both symptoms of inattention and hyperactivity-impulsivity (Appendix B2). Although the DSM-5 criteria for ADHD has since been published, there are no essential differences in the symptoms listed for ADHD between the DSM-IV (APA, 1994) and DSM-5 (APA, 2013) versions. Therefore, the SNAP-IV scale was seen as an appropriate instrument to use in this study. The scale consists of nine items measuring symptoms of inattention and nine items measuring symptoms of hyperactivity-impulsivity. Each of these subsets are summed to obtain total scores for the inattention and hyperactivity-impulsivity symptom domains respectively. A total score for ADHD symptom severity is derived from summing all 18 items. The scale also includes eight items for measuring symptoms of ODD, resulting in a total of 26 items. In the current chapter, only the 18 items measuring symptoms of ADHD were utilised. All items are measured on a four-point Likert scale, ranging from 0 (Not at all) to 3 (Very much).

b) A self-compiled questionnaire gathering biographical information such as gender and age, as well as a question related to whether the child has been diagnosed with ADHD by a healthcare professional (Appendix B1). Parents were also asked whether they experienced any pregnancy and/or delivery complications with the child in question, and if they did, to specify the nature of these complications.

Children of parents who provided consent for their participation in genetics research were asked to provide 1ml of saliva by spitting in a test tube. The test tubes and questionnaires were linked via a numbering system, allowing participation in the study to remain anonymous.

9.2.3 DNA extraction

A high-salt DNA extraction method, first described by (Quinque, Kittler, Kayser, Stoneking, & Nasidze, 2006), was modified to extract DNA from saliva samples. One ml lysis buffer plus 20 µl Proteinase K (20 mg/ml) and 150 µl of 10% SDS were added to each 1 ml saliva sample, where after the samples were incubated overnight at 53°C in a water bath. After incubation, samples were removed from the water bath and 400 µl of 5M NaCl were added to each sample. Samples were then incubated on ice for 10 minutes, and centrifuged
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for 20 minutes at 3 962 g. Hereafter, 1 ml of the supernatant of each sample was distributed into 1.7 ml Eppendorf tubes and 700 µl of isopropanol was added to each tube. The samples in the Eppendorf tubes were placed on an orbital shaker for 10 minutes, where after it was centrifuged at 15 871 g for 15 minutes. The supernatant of each sample was discarded and the pellets washed twice with 500 µl of 70% Ethyl Alcohol on the orbital shaker for 30 minutes. The pellets were left to dry at room temperature for three hours and dissolved in 100 µl nuclease free water in a heating block for one hour at 50 °C. The dissolved DNA was stored at -21°C.

A Nanodrop spectrophotometer was used to quantify the DNA by following the manufacturer’s instructions. All DNA samples were diluted for further analysis to a concentration of 50 ng/µl with nuclease free water.

9.2.4 Genotyping

The 40 base pair VNTR in the 3’ untranslated region of the DAT1 gene was analysed via a polymerase chain reaction (PCR) method, using the following primers (obtained from Whitehead Scientific):

DAT1 forward primer: 5’ – TGCGGTGTAGGGAACGGCCTGAG – 3’

DAT1 reverse primer: 5’ – CTTCCTGGAGGTCACGGCTCAAGG – 3’ (Vandenbergh, Persico, Hawkins, Griffin, Li, Jabs, et al., 1992)

The PCR conditions used were a modification of that previously described by Agudelo, Gálvez, Fonseca, Mateus, Talero-Gutiérrez, and Velez-Van-Meerbeke (2015). Reactions were performed in a total volume of 17 µl containing 150 ng genomic DNA, 6.25 µl Dreamtaq Mastermix (Fermentas Life Sciences), 0.5 µl of each primer at a concentration of 10 µM, 5.25 µl nucleic acid free water (Fermentas Life Sciences) and 2 µl dimethyl sulfoxide at 10%. PCR was carried out using the following conditions: denaturing at 94°C for 5 minutes, followed by 30 cycles of denaturing at 94°C for 45 seconds, annealing at 61°C for 45 seconds and extension at 72°C for 45 seconds, followed by a final extension step at 72°C for 10 minutes.

PCR products were separated electrophoretically on a 3% agarose gel, intercalated with GelRed and visualised under UV light. Genotyping of successfully amplified samples were
performed by first sequencing 10% of the amplified samples (only homozygotes) to accurately determine the number of VNTR repeats present for each. Homozygote samples were sequenced by means of an ABI 3130 Automated Capillary Sequencer. The pre-sequencing PCR was performed in separate forward and reverse total reaction volumes of 20 µl, containing 1 µl PCR product, 12.7 µl nucleic acid free water (Fermentas Life Sciences), 4 µl Ready Reaction Premix (Applied Biosystems Big Dye® Sequencing Kit), 2 µl Big Dye® 5 X Sequencing Buffer (Applied Biosystems Big Dye® Sequencing Kit) and 0.3 µl primer at a concentration of 10 µM (Fermentas Life Sciences). Pre-sequencing PCR was carried out using the following conditions: an initial 1 minute denaturing step at 96°C, followed by 25 cycles at 96°C for 10 seconds, 56°C for 5 seconds and 60°C for 4 minutes, followed by a final extension phase at 72°C for 5 minutes. The sequencing clean-up was conducted by following the ‘ZR DNA Sequencing Clean-Up™ Kit’ protocol. Finally, samples were stored in the dark at 4°C.

Forward and reverse sequencing results were edited and aligned by making use of the BioEdit software programme, version 7.1.9, available from (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The number of repeats of the DAT1 3’ uVNTR was determined by counting the number of times the 40 base pair sequence was repeated for each of the sequenced samples. It is important to note that a number of variations have been identified within the repeat units of the DAT1 3’ uVNTR, with Fuke, Suo, Takahashi, Koike, Sasagawa, and Ishiura (2001) citing nine variations, namely:

(a) AGGAGCGTGCTCTATCCCGGACGCATGCAGGGCCCCCAC
(b) AGGAGCATGTCCTATCCCTGGACGCATGCAGGGCCCCCAC
(c) AGGAGCGTGCTACTACCCAGAACGCATGCAGGGCCCCCAC
(d) AGGAGCGTGCTACTACCCAGACGCATGCAGGGCCCCCAC
(e) TGGAGCGTGCTACTACCCAGACGCATGCAGGGCCCCCAC
(f) AGGAGCGTGCTCTATCCCGGACGCATGCAGGGCCCCCAC
(g) AGGAGCGTGCTACTACCCAGGATGCATGCAGGGCCCCCAC
The remainder of the homozygote samples, as well as the heterozygote samples, were genotyped by comparing their fragment sizes to those of the sequenced homozygote samples on the agarose gels.

9.2.5 Statistical analysis

Data were cleaned prior to analysis and descriptive statistics calculated for all demographic and categorical variables. Mean imputation was used to replace missing values for both the hyperactivity-impulsivity and inattention subscales of the SNAP-IV scale (Siddiqui, 2015). Participants had to have answered at least seven out of the nine (83% of nine – rounded) questions for both the inattention and hyperactivity-impulsivity symptom domains for means to be imputed. Any participants who answered fewer than seven out of nine questions for any one of the subscales were excluded from the inferential statistical analysis. To assure the reliability of the measuring instruments, internal consistency reliability was calculated for both the inattention and hyperactivity-impulsivity subscales of the SNAP-IV scale. Data analysis was conducted through the statistical software package, SPSS version 23, and Microsoft Excel 2016.

An index of pregnancy and/or delivery complications were created by assigning the number one to participants for whom the parents indicated that they experienced either pregnancy and/or delivery complications and the number zero to participants for whom no pregnancy or delivery complications were indicated by the parents. Similarly, the number one was assigned to participants who proved to be homozygous for the 10-repeat allele of the DAT1 3’ uVNTR, whilst the number zero was assigned to 9-repeat homozygous and 9/10 repeat heterozygous participants. Two two-way analysis of variances (two-way ANOVAs) were run to test for a possible interaction effect between the presence of pregnancy and/or delivery complications and the DAT1 3’ uVNTR genotype on symptoms of inattention and hyperactivity-impulsivity respectively.
The significance level for the tests was set at an alpha value of 0.05, and all tests were two-sided (see Appendix E6 for extracts of the statistical analysis).

9.3 Results

The average age of the children in the sample was 10 years with 36 (70.6%) males and 15 (29.4%) females (Table 9.1). Of the 51 children in the sample, 45 (88.2%) were diagnosed with ADHD whilst six (11.8%) participating children did not have the disorder.

*Table 9.1. Demographic characteristics and diagnostic status.*

<table>
<thead>
<tr>
<th>Age (Mean ± SD)</th>
<th>10 ± 3</th>
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</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male [Count(%)]</td>
<td>36 (70.6%)</td>
</tr>
<tr>
<td>Female [Count(%)]</td>
<td>15 (29.4%)</td>
</tr>
<tr>
<td><strong>ADHD diagnoses</strong></td>
<td></td>
</tr>
<tr>
<td>Diagnosed with ADHD [Count(%)]</td>
<td>45 (88.2%)</td>
</tr>
<tr>
<td>Not diagnosed with ADHD [Count(%)]</td>
<td>6 (11.8%)</td>
</tr>
</tbody>
</table>

9.3.1 Reliability of measuring instruments

The SNAP-IV scale showed good internal consistency reliability for both the inattention and hyperactivity-impulsivity subscales, with Cronbach’s alpha coefficient values of 0.938 and 0.928 for the inattention and hyperactivity-impulsivity subscales respectively.

9.3.2 Genotyping results and allelic frequencies

Only the 9 and 10-repeat alleles of the *DAT1* 3’ uVNTR were identified in the current sample through sequencing and comparison with sequenced samples on the agarose gels (Appendix C1 and Appendix C2). Thus, this genomic region is polymorphic in this South African sample. The genotype frequencies were as follows: 10/10 homozygotes = 31.4%; 9/9 homozygotes = 31.4%; and 9/10 heterozygotes = 37.3%.

The two alleles were observed in equal proportions (frequency = 0.5 for both the 9- and 10-repeat alleles).
9.3.3 Two-way ANOVA results

**DAT1 3’ uVNTR genotype as a moderator of the impact of pregnancy and delivery complications on inattention symptom severity**

A two-way ANOVA was run to determine whether the influence of pregnancy and delivery complications on inattention symptom severity is dependent on DAT1 3’ uVNTR genotype. As explained earlier, the 10/10 homozygote genotype was considered the risk genotype. All assumptions of the two-way ANOVA were satisfied, including the absence of extreme outliers, normally distributed residuals, and homogeneity of variances (Table 9.2).

Table 9.2. Two-way ANOVA results – Inattention as dependent variable.

<table>
<thead>
<tr>
<th></th>
<th>Type III Sum of Squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>300.583</td>
<td>3</td>
<td>100.194</td>
<td>2.133</td>
<td>0.109</td>
<td>0.120</td>
</tr>
<tr>
<td>Intercept</td>
<td>15999.226</td>
<td>1</td>
<td>15999.226</td>
<td>340.538</td>
<td>0.000</td>
<td>0.879</td>
</tr>
<tr>
<td>DAT 10 Repeat (Homozygous)</td>
<td>76.934</td>
<td>1</td>
<td>76.934</td>
<td>1.638</td>
<td>0.207</td>
<td>0.034</td>
</tr>
<tr>
<td>Pregnancy and/or delivery complications</td>
<td>77.821</td>
<td>1</td>
<td>77.821</td>
<td>1.656</td>
<td>0.204</td>
<td>0.034</td>
</tr>
<tr>
<td>DAT 10 Repeat (Homozygous) * Pregnancy and/or delivery complications</td>
<td>281.165</td>
<td>1</td>
<td>281.165</td>
<td>5.984</td>
<td>0.018</td>
<td>0.113</td>
</tr>
<tr>
<td>Error</td>
<td>2208.163</td>
<td>47</td>
<td>46.982</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21225.000</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>2508.745</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There were no significant main effects for either the DAT1 3’ uVNTR genotype, or pregnancy and/or delivery complications on inattention symptom severity scores (Table 9.2). However, there was a significant interaction effect between these two variables on inattention symptom severity scores, $F(1, 47) = 5.984, p = 0.018$, partial $\eta^2 = 0.113$. Thus, the effect of pregnancy and/or delivery complications on inattention symptom severity did
Interaction between DAT 1 3’ and pregnancy complications on ADHD symptoms depend on the DAT1 3’ uVNTR genotype carried.

To explore this interaction effect further, a simple effects analysis was run. This analysis studies the effect of one independent variable on the dependent variable within the different levels of the other independent variable (Field, 2013). Thus, the simple effects analysis run here explored the effect of pregnancy and/or delivery complications on inattention symptom severity scores separately for the two DAT1 3’ uVNTR genotype groups. This analysis revealed a statistically significant effect for pregnancy and/or delivery complications on inattention symptom severity scores, but only for individuals homozygous for the 10-repeat allele of the DAT1 3’ uVNTR, F(1, 47) = 4.854, p = 0.033, partial η2 = 0.094. In contrast, 9-repeat homozygotes or 9/10 heterozygotes who were exposed to pregnancy and/or delivery complications did not show statistically significant differences in their mean inattention symptom severity scores (Mean = 17.471) when compared to the same allele carriers not exposed to pregnancy and/or delivery complications (Mean = 20), F(1, 47) = 1.191, p = 0.281, partial η2 = 0.025.

Individuals homozygous for the 10-repeat allele who had been exposed to pregnancy and/or delivery complications had significantly higher inattention symptom severity scores (Mean = 25.600) than 10-repeat homozygotes who were not exposed to pregnancy and/or delivery complications (Mean = 17.455).

**DAT1 3’ uVNTR genotype as a moderator of the impact of pregnancy and delivery complications on hyperactivity-impulsivity symptom severity**

A two-way ANOVA (Table 9.3) was run to determine whether the influence of pregnancy and/or delivery complications on hyperactivity-impulsivity symptom severity is dependent on DAT1 3’ uVNTR genotype. All assumptions of the two-way ANOVA were satisfied, including the absence of extreme outliers, normally distributed residuals, and homogeneity of variances.
Interaction between DAT 1 3’ and pregnancy complications on ADHD symptoms / 181

Table 9.3. Two-way ANOVA results – hyperactivity-impulsivity as dependent variable.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>Type III Sum of Squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>424.933</td>
<td>3</td>
<td>141.644</td>
<td>2.120</td>
<td>0.110</td>
<td>0.119</td>
</tr>
<tr>
<td>Intercept</td>
<td>10639.020</td>
<td>1</td>
<td>10639.020</td>
<td>159.234</td>
<td>0.000</td>
<td>0.772</td>
</tr>
<tr>
<td>DAT 10 Repeat (Homozygous)</td>
<td>0.220</td>
<td>1</td>
<td>0.220</td>
<td>0.003</td>
<td>0.954</td>
<td>0.000</td>
</tr>
<tr>
<td>Pregnancy and/or delivery complications</td>
<td>273.243</td>
<td>1</td>
<td>273.243</td>
<td>4.090</td>
<td>0.049</td>
<td>0.080</td>
</tr>
<tr>
<td>DAT 10 Repeat (Homozygous) * Pregnancy and/or delivery complications</td>
<td>283.175</td>
<td>1</td>
<td>283.175</td>
<td>4.238</td>
<td>0.045</td>
<td>0.083</td>
</tr>
<tr>
<td>Error</td>
<td>3140.244</td>
<td>47</td>
<td>66.814</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16240.000</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>3565.176</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was a significant main effect for pregnancy and/or delivery complications on hyperactivity-impulsivity symptom score severity, $F(1, 47) = 4.090, p = 0.049$, partial $\eta^2 = 0.080$, but not for the DAT1 3’ uVNTR genotype (Table 9.3). As was the case for inattention, there was a significant interaction effect between DAT1 3’ uVNTR genotype and pregnancy and/or delivery complications on hyperactivity-impulsivity symptom severity scores, $F(1, 47) = 4.238, p = 0.045$, partial $\eta^2 = 0.083$. Thus, the effect of pregnancy and/or delivery complications on hyperactivity-impulsivity symptom severity did depend on the DAT1 3’ uVNTR genotype carried. The main effect of pregnancy and/or delivery complications on symptom severity was not explored further, since the significant interaction effect shows that this main effect is moderated by the DAT1 3’ uVNTR genotype carried.

To explore this interaction effect further, a simple effects analysis was run to analyse the effects of pregnancy and/or delivery complications on hyperactivity-impulsivity symptom
severity within each of the DAT1 3’ uVNTR genotype groups. As was the case for inattention, this analysis revealed a statistically significant effect for pregnancy and/or delivery complications on hyperactivity-impulsivity symptom severity scores, but only for individuals homozygous for the 10-repeat allele of the DAT1 3’ uVNTR, F(1, 47) = 5.801, p = 0.020, partial η² = 0.110. In contrast, 9-repeat homozygotes or 9/10 heterozygotes who were exposed to pregnancy and/or delivery complications did not show statistically significant differences in their mean symptom severity scores (Mean = 16.294) when compared to the same allele carriers not exposed to pregnancy and/or delivery complications (Mean = 16.389), F(1, 47) = 0.001, p = 0.973, partial η² = 0.000.

Again, similar to the results observed for inattention, individuals homozygous for the 10-repeat allele who had been exposed to pregnancy and/or delivery complications had significantly higher hyperactivity-impulsivity symptom severity scores (Mean = 21.800) than 10-repeat homozygotes who were not exposed to pregnancy and/or delivery complications (Mean = 11.182).

9.4 Discussion

The aim of this study was to determine whether the impact of pregnancy and/or delivery complications on ADHD symptom severity depended on the alleles carried in the DAT1 3’ uVNTR. Due to the non-probability sampling strategy employed, the sample was not representative of the South African population, and results should therefore not be generalized to the South African population at large. Non-representative samples are common in behavioural genetic research (e.g. Agudelo, Gálvez, Fonseca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Van Dyk, Springer, Kidd, Steyn, Solomons, & Van Toorn, 2014), but nonetheless limits the results of this study to the sample under investigation. A significant interaction effect between the DAT1 3’ uVNTR genotype and pregnancy and/or delivery complications would provide a plausible explanation for the conflicted findings reported for the impact of the DAT1 3’ uVNTR on ADHD in the literature thus far (Faraone, & Mick, 2010; Gatt, Burton, Williams, & Schofield, 2015; Gizer, Ficks, & Waldman, 2009).
The finding that the 9- and 10-repeat alleles of the DAT1 3’ uVNTR were the only alleles present in the current study is in line with previous research showing these two alleles to be the most frequently observed (Agudelo, Gálvez, Fonseca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Doucette-Stamm, Blakely, Tian, Mockus, & Mao, 1995; Gizer, Ficks, & Waldman, 2009; Kang, Palmatier, & Kidd, 1999; Santovito, Cervella, Selvaggi, Caviglia, Burgarello, Sella, et al., 2008). The significant interaction effect found between the DAT1 3’ uVNTR and pregnancy and/or delivery complications on both inattention and hyperactivity-impulsivity symptom severity supports the hypothesis put forth by Faraone, and Mick (2010) and Gizer, Ficks, and Waldman (2009) that the conflicted findings may be due to the interaction of this polymorphism with factors in the environment. As was found in the meta-analysis by Gizer, Ficks, and Waldman (2009), the 10-repeat allele of the DAT1 3’ uVNTR also proved to be the risk allele in the current study. Pregnancy and/or delivery complications resulted in higher inattention and hyperactivity-impulsivity symptom severity scores only in individuals homozygous for the 10-repeat allele. The lack of previous studies examining a possible interaction effect between the DAT1 3’ uVNTR and pregnancy and/or delivery complications on ADHD symptom severity makes placing this finding in context difficult. The studies conducted to date on a possible interaction effect were limited to exploring the interaction between the DAT1 3’ uVNTR and low birth weight only (Jackson, & Beaver, 2015; Langley, Turic, Rice, Holmans, Van den Bree, Craddock, et al., 2008). The current finding is in line with the results from the Jackson, and Beaver (2015) study, where the 10-repeat allele together with low birth weight resulted in an increased risk of ADHD symptoms, but is contradictory to the Langley, Turic, Rice, Holmans, Van den Bree, Craddock, et al. (2008) study where no association could be found.

The findings of no main effect for the DAT1 3’ uVNTR on either inattention or hyperactivity-impulsivity is congruent with the results reported in the meta-analysis by Li, Sham, Owen, and He (2006), and adds to the already large body of conflicting literature on the influence of this polymorphism on ADHD. This lack of a main effect, but the presence of an interaction effect, points to a possible gene-environment interaction effect being at play. Although the mechanisms through which environmental factors interact with genes to influence ADHD is not well understood, evidence is accumulating that epigenetic processes may be involved (Gervin, Nordeng, Ystrom, Reichborn-Kjennerud, & Lyle, 2017; Van Mil,
Steegers-Theunissen, Bouwland-Both, Verbiest, Rijlaarsdam, Hofman, et al., 2014; Walton, Pingault, Cecil, Gaunt, Relton, Mill, et al., 2017; Xu, Chen, Luo, Tang, Zhang, Wu, et al., 2015), presenting a promising avenue for future research. The conflicted findings for the influence of the \textit{DAT1} 3’ uVNTR may therefore be due to this polymorphism not directly influencing ADHD symptom severity, but rather moderating the influence of pregnancy and/or delivery complications on ADHD symptom severity.

9.5 Conclusion

The results from this study show that there might be a possible gene-environment interaction effect at play between the \textit{DAT1} 3’ uVNTR and pregnancy and/or delivery complications on inattention and hyperactivity-impulsivity symptom severity. Pregnancy and/or delivery complications influenced inattention and hyperactivity-impulsivity symptom severity, but only in individuals homozygous for the 10-repeat allele of the \textit{DAT1} 3’ uVNTR. This significant interaction effect may provide an explanation for the conflicted findings reported in the literature thus far for the influence of the \textit{DAT1} 3’ uVNTR on ADHD. It suggests that rather than directly influencing ADHD symptom severity, this polymorphism moderates the influence of an environmental factor on ADHD symptom severity. This study was also the first of its kind to study a possible interaction effect between the \textit{DAT1} 3’ uVNTR and pregnancy and/or delivery complications in a South African sample, and may serve as a basis for future research in this country. Worldwide, more research should be conducted looking at the influence of interactions between the \textit{DAT1} 3’ uVNTR genotype and pregnancy and delivery complications, other than maternal substance use, on ADHD symptom severity.

9.6 Limitations of the study

This study had a number of limitations that should be highlighted. Of importance is the small sample size, resulting in small subgroupings of participants within the different level combinations of the two independent variables. The small sample size was due to the limited funds available for the considerable costs involved in molecular genetic analysis of samples. This also resulted in the sample not being aggregated by gender or by the simplex/multiplex distinction of the disorder for the analysis, since the number of individuals in each cell of the statistical design would have been too small. Given the findings in Chapter 7 of this thesis...
where a possible divergence between male and female ADHD was shown, as well as the findings in Chapter 8 where evidence was provided for the validity of distinguishing between simplex and multiplex ADHD, further aggregating the sample by these factors in molecular genetic analysis of the disorder may be informative and should be considered for future research. Due to the small sample size, only five participants were homozygous for the 10-repeat allele of the DAT1 3’ uVNTR and were exposed to pregnancy and/or delivery complications. The finding that individuals in this subcategory have higher inattention and hyperactivity/impulsivity symptom severity scores therefore needs to be replicated in future studies with larger sample sizes.

A further limitation is that, due to the exploratory nature of the study, only age and adoption status were used as exclusion criteria. Stricter exclusion criteria should be considered in future research attempting to replicate these results. In addition, this study relied on parent-reported ADHD symptom severity scores only. Since combining information from multiple informants has been shown to improve validity (Martel, Schimmack, Nikolas, & Nigg, 2015), future studies should try to obtain information on participants’ symptom severity from more than one source. Although diagnostic classification was only used for descriptive purposes, and diagnostic status was confirmed for individuals diagnosed with ADHD, parent-report only was relied upon to classify individuals as not diagnosed with ADHD. Although preferable, due to cost and time limitations, structured diagnostic interviews performed on all participants directly by healthcare professionals were not feasible for this study. That said, lending some credence to the parent-report methodology used here, a study by Visser, Danielson, Bitsko, Perou, and Blumberg (2013) showed that the prevalence of ADHD as gleaned from parent-report of diagnosis by a healthcare professional was statistically indistinguishable from the prevalence of ADHD as obtained directly from medical records of healthcare providers.

A final limitation to this study was that, due to the non-probability sampling strategy employed, the sample was not representative of the South African population. Although, as noted, this is common in published studies in behavioural genetic research, this does preclude the generalization of findings to the South African population at large.
Chapter 10

Testing for the presence of rare sequence variations within the MAOA-uVNTR in a sample of children diagnosed with Attention-Deficit/Hyperactivity Disorder (ADHD)

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This chapter is the second chapter in the thesis to explore the molecular genetic architecture of Attention-Deficit/Hyperactivity Disorder (ADHD). A possible reason for the mixed findings is that rare genetic variants within well-known polymorphisms play a role in the disorder. In this chapter, a common variable number of tandem repeats polymorphism in the monoamine oxidase A gene is explored for the presence of possible rare variants in a sample of children diagnosed with ADHD in South Africa. The research conducted, the statistical analysis, and the writing of this chapter was done by Nadia Fouché.

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**Abstract**

In line with the common disease/common variant hypothesis, a number of studies have been conducted on the role of common repeat variants of the variable number of tandem repeats polymorphism in the promoter region of the monoamine oxidase A gene (MAOA-uVNTR). Results from these studies have been conflicting, with some showing that the MAOA-uVNTR is associated with ADHD and others finding no such association. One possible explanation for the mixed findings is that, in line with the common disease/rare variant hypothesis, rare sequence variations within the 30 base pair repeat sequence influences ADHD, and not just the number of repeats present. Therefore, the aim of the current study was to determine whether any rare sequence variations within the 30 base pair repeat sequence of the MAOA-uVNTR could be detected in a sample of South African children diagnosed with ADHD. The final sample consisted of 33 children, all diagnosed with ADHD. Results showed that, although the vast majority of diagnosed children only carried the common 30 base pair wild type repeat sequence, two siblings carried a rare deletion of 12 base pairs at the tail-end of the fourth repeat of the 30 base pair sequence. Although still preliminary, this finding shows that it may be worthwhile for future research to not only look at the number of repeats of a VNTR polymorphism in relation to ADHD, but also to examine sequence variations within the repeat sequences.

**Keywords** Attention-Deficit/Hyperactivity Disorder, Common disease/common variant hypothesis, Common disease/rare variant hypothesis, Monoamine oxidase A gene, Rare genetic variants, Variable number of tandem repeats polymorphism
10.1 Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is one of the most frequently occurring childhood disruptive behavioural disorders and is characterised by symptoms of inattention and/or hyperactivity-impulsivity (American Psychiatric Association [APA], 2013; Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007). ADHD has been shown to be highly heritable, with a meta-analytic study showing that genetic factors account for up to 70% of the variance for both the inattention and hyperactivity-impulsivity symptom domains (Nikolas, & Burt, 2010).

Numerous studies have been conducted on the effect of common genetic variants on ADHD (Faraone, Perlis, Doyle, Smoller, Goralnick, Holmgren, et al., 2005; Gizer, Ficks, & Waldman, 2009; Stergiakouli, Hamshere, Holmans, Langley, Zaharieva, deCODE Genetics, et al., 2012). Results from these studies have, however, been highly conflicting. These studies are all based on the common disease/common variant (CDCV) hypothesis which states that high frequency diseases in the human population can be attributed to equally common genetic variations (Collins, 1997; Gibson, 2012; Schork, Murray, Frazer, & Topol, 2009). However, the high rate of conflicting results in studies of common genetic variants influencing ADHD alludes to these variants not being the only genetic factors influencing the disorder.

One possible explanation for the mixed findings stems from an opposing hypothesis, known as the common disease/rare variant (CDRV) hypothesis. This hypothesis states that a multitude of rare, mildly deleterious genetic variations with relatively high penetrance are the major underlying factors in common human disease (Pritchard, 2001; Schork, Murray, Frazer, & Topol, 2009; Smith, 2002; Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012). In the case of ADHD, both common and rare variants seem to be involved in the aetiology of the disorder, rather than just one of the two (Martin, O’Donovan, Thapar, Langley, & Williams, 2015; Stergiakouli, Hamshere, Holmans, Langley, Zaharieva, deCODE Genetics, et al., 2012). Assuming that the occurrence of ADHD can be explained by a polygenic liability threshold model, Martin, O’Donovan, Thapar, Langley, and Williams (2015) studied how common and rare genetic variants jointly contribute to the aetiology of ADHD. Aggregate scores of common genetic variants implicated in the aetiology of ADHD (named
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‘polygenic risk scores’) were compared between diagnosed individuals with and without rare risk alleles. The researchers found that individuals diagnosed with ADHD who carried rare risk alleles had lower polygenic risk scores for ADHD than diagnosed children without rare variants. This finding is in line with the CDRV hypothesis which associates rare genetic variants with high penetrance. Thus, it is possible that rare variants are an independent and alternative cause of ADHD, overcoming the disease liability threshold in the absence of common genetic risk variants due to high penetrance. Mixed findings in a study of common variants may be the result of heterogenous samples (sample consists of individuals diagnosed with ADHD, with either a rare variant or with only the common risk variant).

One form of rare genetic variant for which research is lacking is DNA sequence variations within commonly observed repeat motifs of variable number of tandem repeat (VNTR) polymorphisms. To date, a limited number of studies have focused on sequence variations within repeat motifs of VNTR’s in relation to ADHD (Grady, Chi, Ding, Smith, Wang, Schuck, et al., 2003; Hawi, Cummins, Tong, Johnson, Lau, Samarrai, et al., 2015; Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012; Tovo-Rodrigues, Rohde, Menezes, Polanczyk, Kieling, Genro, et al., 2013). The few studies that have been conducted focused exclusively on the 48 base pair VNTR in exon three of the DRD4 gene (Grady, Chi, Ding, Smith, Wang, Schuck, et al., 2003; Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012; Tovo-Rodrigues, Rohde, Menezes, Polanczyk, Kieling, Genro, et al., 2013). Subsequent findings unanimously indicated that there is an excess of rare haplotype variants of the DRD4 exon three VNTR in ADHD probands. This highlights the importance of studying the DNA sequences of the repeat motifs of VNTR polymorphisms, along with the number of repeats.

A VNTR polymorphism that has been the subject of many research studies in behavioural genetics is the VNTR in the promotor region of the monoamine oxidase A gene (MAOA), first described by Sabol, Hu, and Hamer (1998). The MAOA gene has been mapped to the short arm of the X chromosome (Xp11.3) (Levy, Powell, Buckle, Hsu, Breakefield, & Craig, 1989). This gene codes for the monoamine oxidase A enzyme (MAOA), which has been found to catalyse the degradation of biogenic amines including dopamine (Bortolato, Chen, & Shih, 2008; Chen, Hotamisligil, Huang, Wen, Ezzeddine, Aydin-Muderrisoglu, et al., 1991). Evidence linking this gene to ADHD mainly comes from a family study conducted by Brunner,
Nelen, Breakefield, Ropers, and van Oost (1993). In this study, the researchers found a point mutation in the eighth exon of the MAOA gene in a large Dutch family which resulted in complete and selective deficiency in MAOA enzymatic activity in affected males in this family. Various impulsive behaviours were noted in the affected males, including aggressive outbursts, arson, attempted rape, and exhibitionism. In addition, medication that inhibits MAOA activity has shown some success in the treatment of children with ADHD (Bonnet, 2003), implicating a possible role for MAOA in the disorder.

The promotor VNTR in the MAOA gene (the MAOA-uVNTR) is located 1.2kb upstream of the MAOA coding sequences and consists of a 30 base pair sequence that is repeated a variable number of times (Sabol, Hu, & Hamer, 1998). This polymorphism has been shown to be functional, with the number of copies carried by an individual influencing the transcriptional efficiency of the MAOA gene (Deckert, Catalano, Syagailo, Bosi, Okladnova, Di Bella, et al., 1999; Sabol, Hu, & Hamer, 1998). In addition, this polymorphism has been shown to be present in 2, 3, 3.5, 4 and 5 copies of the 30 base pair repeat sequence (Choi-Kwon, Ko, Jun, Kim, Cho, Nah, et al., 2017; Gizer, Ficks, & Waldman, 2009), with the 3 and 4 repeat alleles being the most frequently observed (Ficks, & Waldman, 2014; Hung, Lung, Hung, Chong, Wu, Wen, et al., 2012). The first study to examine the association between the MAOA-uVNTR and ADHD was conducted by Manor, Tyano, Mel, Eisenberg, Bachner-Melman, Kotler, et al. (2002). Results pointed to the longer alleles of this polymorphism (3.5, 4 and 5 repeats) conveying risk for ADHD. Making use of this initial finding, Gizer, Ficks, and Waldman (2009) designated these longer alleles as the risk alleles in their meta-analysis. Pooling data from six studies, latter researchers found no significant association between ADHD and these risk alleles. Following the results of this meta-analysis, few subsequent studies looking at a main effect of the MAOA-uVNTR on ADHD have been conducted. One study conducted by El-Tarras and colleagues in 2012 showed a positive association between the 3/4 and 3/2 MAOA-uVNTR genotypes and ADHD in a sample from Saudi Arabia (El-Tarras, Alsulaimani, Awad, Mitwaly, Said, & Sabry, 2012). It is thus clear that no consensus has been reached regarding the role of the MAOA-uVNTR in ADHD, with studies yielding conflicting findings.
To the current researchers’ knowledge, no studies have been conducted on possible rare sequence variations within the MAOA-uVNTR influencing ADHD. Previous research conducted on an association between this polymorphism and ADHD have exclusively focused on the number of repeats present. Thus, the aim of the current study is to determine whether any rare mutations within the MAOA-uVNTR are present in a sample of children diagnosed with ADHD. Such rare mutations, should they be present, could provide an explanation for the conflicting results found thus far for the role of the MAOA-uVNTR in ADHD.

10.2 Methods
10.2.1 Participants

The final sample consisted of 33 children, all diagnosed with ADHD. For all participants in this chapter, parent-reported diagnosis of ADHD was directly confirmed by the healthcare professionals who made the diagnoses. Girls heterozygous for the MAOA-uVNTR, children for whom DNA did not amplify, as well as children not diagnosed with ADHD were excluded from the final sample.

10.2.2 Procedure

The sample was recruited through social media pages via the Attention Deficit and Hyperactivity Support Group of Southern Africa (ADHASA) as well as through medical professionals working with children diagnosed with ADHD in the Bloemfontein area, Free State Province of South Africa. Parents were provided with two information leaflets, one describing the study (Appendix A1), and the other providing information on genetic research (Appendix A3). In addition, parents were provided with two informed consent forms to sign. One for consenting to complete a biographical questionnaire for their children (Appendix A2) and the other for consenting to allow their children to participate in genetics research (Appendix A4).

The biographical questionnaire was self-compiled and consisted of questions related to the child’s gender and age, as well as a question related to whether the child has been diagnosed with ADHD by a healthcare professional (Appendix B1). Children for whom

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4 Paediatrician: Prof Andre Venter; Clinical psychologist: Lida van Zyl
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parents provided consent to participate in genetic research were asked to provide 1 ml of saliva by spitting in a test tube. The test tubes and questionnaires were linked via a numbering system, allowing participation in the study to remain anonymous.

10.2.3 DNA extraction

A high-salt DNA extraction method, first described by Quinque, Kittler, Kayser, Stoneking, and Nasidze (2006), was modified to extract DNA from the saliva samples. One millilitre lysis buffer, plus 20 µl Proteinase K (20 mg/ml) and 150 µl of 10% SDS were added to each 1 ml saliva sample, where after the samples were incubated overnight at 53°C in a water bath. After incubation, samples were removed from the water bath, and 400 µl of 5M NaCl were added to each sample. Samples were then incubated on ice for 10 minutes and centrifuged for 20 minutes at 3 962 g. Hereafter, 1 ml of the supernatant of each sample was distributed into 1.7 ml Eppendorf tubes and 700 µl of isopropanol was added to each tube. The samples in the Eppendorf tubes were placed on an orbital shaker for 10 minutes, where after it was centrifuged at 15 871 g for 15 minutes. The supernatant of each sample was discarded and the pellets washed twice with 500 µl of 70% Ethyl Alcohol on the orbital shaker for 30 minutes. The pellets were left to dry at room temperature for three hours and dissolved in 100 µl nuclease free water in a heating block for one hour at 50°C. The dissolved DNA was stored at -21°C.

A Nanodrop spectrophotometer was used to quantify the DNA by following the manufacturer’s instructions. All DNA samples were diluted for further analysis to a concentration of 50 ng/µl with nuclease free water.

10.2.4 Genotyping

The 30 base pair VNTR in the promoter region of the MAOA gene was analysed via a polymerase chain reaction (PCR) method, using the following primers (obtained from Whitehead Scientific):

MAOA forward primer: 5’ – ACAGCCTGACCGTGGAGAAG – 3’

The PCR conditions used were a modification of that previously described by Agudelo,
Gálvez, Fonseca, Mateus, Talero-Gutiérrez, and Velez-Van-Meerbeke (2015). Reactions were performed in a total volume of 20 µl containing 100 ng genomic DNA, 10 µl Dreamtaq Mastermix (Fermentas Life Sciences), 0.5 µl of each primer at a concentration of 10 µM, and 7 µl nucleic acid free water (Fermentas Life Sciences). PCR was carried out using the following conditions: denaturing at 95°C for 3 minutes, followed by 35 cycles of denaturing at 95°C for 1 minute, annealing at 64°C for 1 minute and extension at 72°C for 1 minute, followed by a final extension cycle at 72°C for 5 minutes.

PCR products were separated electrophoretically on a 3% agarose gel, intercalated with GelRed and visualised under UV light. Hereafter, only homozygote samples of children diagnosed with ADHD were sequenced to scan the repeat region of the gene for possible sequence variations. Sequencing was done by means of an ABI 3130 Automated Capillary Sequencer. The pre-sequencing PCR was performed in separate forward and reverse total reaction volumes of 10 µl, containing 1 µl PCR product, 4.3 µl nucleic acid free water (Fermentas Life Sciences), 0.5 µl Ready Reaction Premix (Applied Biosystems Big Dye® Sequencing Kit), 1 µl Big Dye® 5 X Sequencing Buffer (Applied Biosystems Big Dye® Sequencing Kit), and 3.2 µl primer at a concentration of 10 µM (Fermentas Life Sciences). Pre-sequencing PCR was carried out using the following conditions: an initial 3 minute denaturing step at 94°C, followed by 25 cycles at 94°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 minutes, followed by a final extension phase at 72°C for 5 minutes. The sequencing clean-up was conducted by following the ‘ZR DNA Sequencing Clean-Up™ Kit’ protocol. Finally, samples were stored in the dark at 4°C.

Forward and reverse sequencing results were edited and aligned by making use of the BioEdit software programme, version 7.1.9, available from (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The number of repeats of the MAOA-uVNTR was determined by counting the number of times the following 30 base pair sequence was repeated for each of the sequenced samples: ACCGGCACCGGCACCAGTACCCGCACCAGT. Hereafter, this 30 base pair sequence was scrutinised for each sample to determine if any sequence variations were present.
10.2.5 Statistical analysis

Data were cleaned prior to analysis and descriptive statistics calculated for the questions in the biographical questionnaire.

10.3 Results

The average age of the children in the sample was 9.4 years, with 29 (87.9%) males and four (12.1%) females (Table 10.1). All children were diagnosed with ADHD by a healthcare professional according to parent-report.

Table 10.1. Demographic characteristics.

<table>
<thead>
<tr>
<th>Age (Mean ± SD)</th>
<th>9.4 ± 3</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male (Count(%))</td>
<td>29 (87.9%)</td>
</tr>
<tr>
<td>Female (Count(%))</td>
<td>4 (12.1%)</td>
</tr>
</tbody>
</table>

10.3.1 Sequencing results and allele frequencies (Appendix D1 and Appendix D2)

Of the four females in the sample, three (75%) were homozygous for the 4.5 repeat allele of the MAOA-uVNTR, whilst one (25%) was homozygous for the 3.5 repeat allele. For the males, 12 (41.4%) were hemizygous for the 3.5 repeat allele of the MAOA-uVNTR, 14 (48.3%) were hemizygous for the 4.5 repeat allele, and one (3.4%) was hemizygous for the 5.5 repeat allele. Sequencing results for all the aforementioned participants showed that only the wild-type sequence of the repeat was carried, namely:

ACCGGCACCGGCACCAGTACCCGCACCAGT

The 3.5 repeat allele therefore had an allele frequency in the total sample of 14/37 (0.38), whereas the 4.5 repeat allele had an allele frequency of 20/37 (0.54) and the 5.5 repeat allele an allele frequency of 1/37 (0.03).

Apart from the common alleles, two siblings diagnosed with ADHD both carried a rare mutation within the above repeat sequence. Both siblings carried three full repeats of the common 30 base pair sequence. However, the fourth repeat in both siblings showed a deletion of the last 12 base pairs of the common repeat sequence (deletion of
MAOA-uVNTR variations in a sample of children with ADHD

ACCGCACCAGT), followed by the same half repeat seen in all the other samples. Due to the rare nature of this result, these two samples were re-sequenced and the finding confirmed. The common 4.5 repeat found in this study is aligned with this rare variant in Figure 10.1.

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Figure 10.1 Comparison between the 4.5 repeat and the rare variant.
*Note: The full repeat regions are highlighted consecutively in red and blue, and the half-repeat at the end in green. Red stars from position 114-125 indicate the deletion.

10.4 Discussion

The first salient finding of the current study was that the repeat lengths that were found for the MAOA-uVNTR differed from that reported in previous studies. In the current study, alleles that carried 3.5, 4.5 and 5.5 repeats of the 30 base pair repeat sequence were found. In contrast, previous studies reported on here all described 3, 3.5, 4 and 5 repeats of the 30 base pair repeat sequence (Deckert, Catalano, Syagailo, Bosi, Okladnova, Di Bella, et al., 1999; El-Tarras, Alsulaimani, Awad, Mitwaly, Said, & Sabry, 2012; Gizer, Ficks, & Waldman, 2009; Manor, Tyano, Mel, Eisenberg, Bachner-Melman, Kotler, et al., 2002; Sabol, Hu, & Hamer, 1998). Further investigation revealed that this discrepancy has been highlighted in only one previous study, conducted by Das, Das, Sinha, Chattopadhyay, Chaudhuri, Singh, et al. (2006). In an Indian sample, latter researchers also found repeat lengths of 2.5, 3.5, 4.5, and 5.5 repeats. They noted that in an accession to Genbank of this VNTR (Genbank accession number M89636), Sabol, Hu, and Hamer (1998) wrongly labelled the 4.5 repeat allele of the 30 base pair VNTR as consisting of only 4 repeats. The vast majority of subsequent studies followed the norm established by Sabol, Hu, and Hamer (1998), and consequently reported the repeat numbers incorrectly. Therefore, similar to the conclusion drawn by Das, Das, Sinha, Chattopadhyay, Chaudhuri, Singh, et al. (2006), we conclude that the 3.5, 4.5, and 5.5
repeats found in our sample correspond to the 3, 4, and 5 repeats described by Sabol, Hu, and Hamer (1998), and subsequently by the other researchers mentioned in this study (Deckert, Catalano, Syagailo, Bosi, Okladnova, Di Bella, et al., 1999; El-Tarras, Alsulaimani, Awad, Mitwaly, Said, & Sabry, 2012; Gizer, Ficks, & Waldman, 2009; Manor, Tyano, Mel, Eisenberg, Bachner-Melman, Kotler, et al., 2002).

Allele frequencies corresponded to that previously reported, with the 3.5 and 4.5 repeat alleles (corresponding to the 3 and 4 repeat alleles in the mentioned studies) being the most common (Ficks, & Waldman, 2014; Hung, Lung, Hung, Chong, Wu, Wen, et al., 2012). The 5-repeat allele was only present in one individual, whilst the 2-repeat allele was not observed in this study. It should be noted that due to the non-probability sampling procedure followed, the sample was not representative of the South African population. Although this is common in published studies in behavioural genetic research (e.g. Agudelo, Gálvez, Fonseca, Mateus, Talero-Gutiérrez, & Velez-Van-Meerbeke, 2015; Biederman, Petty, Hammerness, Woodworth, & Faraone, 2013; Van Dyk, Springer, Kidd, Steyn, Solomons, & Van Toorn, 2014), it nonetheless precludes generalization of the findings to the South African population at large.

Regarding sequence variations within the 30 base pair repeat sequence, the vast majority of participants carried the wild-type sequence. Should the MAOA-uVNTR prove to play a role in ADHD, as suggested by Manor, Tyano, Mel, Eisenberg, Bachner-Melman, Kotler, et al. (2002) and El-Tarras, Alsulaimani, Awad, Mitwaly, Said, and Sabry (2012), this lack of sequence variation within the repeat region will support the common disease/common variant hypothesis (Collins, 1997; Gibson, 2012; Schork, Murray, Frazer, & Topol, 2009). The frequently occurring disorder will then be associated with the frequently reported genetic variations seen for the MAOA-uVNTR.

That said, in line with the common disease/rare variant hypothesis (Pritchard, 2001; Schork, Murray, Frazer, & Topol, 2009; Smith, 2002; Tovo-Rodrigues, Rohde, Roman, Schmitz, Polanczyk, Zeni, et al., 2012), we also found a rare mutation within the 30 base pair repeat sequence in two siblings diagnosed with ADHD. The siblings both carried three full repeats of the wild-type sequence, but then showed a 12 base pair deletion at the tail end of the fourth repeat, followed by a half repeat. Although unpublished direct submissions of this
deletion have been made to GenBank by Mukhopadhyay, Das, and Das (2005) (GenBank accession number DQ314615) and by Gelbmann, Schaschl, and Steinborn (2016) (GenBank accession number KT428698), this mutation has never been described in the literature, either for ADHD or in general populations. The mutation has also never been associated with ADHD.

It is important to note that this finding does not prove that this mutation within the MAOA-uVNTR can either cause or be associated with ADHD. More research in larger samples of children diagnosed with ADHD is needed to establish whether this mutation is present in a significant number of children with the disorder. However, should future research show this mutation to indeed be associated with ADHD, it would lend credence to the CDRV hypothesis that posits that rare mutations with relatively high penetrance play a role in commonly observed disorders. That said, findings from the current study show that, even if this mutation is found to be associated with ADHD, it cannot play a role in ADHD in all diagnosed individuals. A clear majority of children who have been diagnosed with ADHD in the current sample do not carry this mutation. It is therefore possible that rare genetic variants such as this play a role in only a subset of diagnosed individuals. This could lead to mixed findings should a study sample be heterogeneous for individuals carrying common mutations and those carrying rare mutations associated with the disorder.

10.5 Conclusion

In conclusion, the aim of this study was to determine whether rare variations within the MAOA-uVNTR sequence could be found in a sample of children diagnosed with ADHD. A rare deletion at the tail-end of the commonly observed repeat sequence was indeed found in two siblings diagnosed with the disorder. More research is needed to determine whether this mutation can indeed be associated with ADHD. The findings in this study show that it might be worthwhile for future researchers to focus on sequence variations within repeat regions of VNTR polymorphisms, instead of just focusing on the number of repeats present, when searching for molecular genetic causes of ADHD.

10.6 Limitations of the study

There were several limitations to this study. Firstly, the small sample size hindered the
researchers from drawing conclusions regarding the role of the mutation found in ADHD. Future studies should aim to see whether this mutation is found frequently in the ADHD population. In addition, the sample was not representative of the South African population at large. Although this is common in behavioural genetic research (as noted), this does mean that results regarding gene frequencies should not be generalized to the population. A further limitation is that heterozygote females were not included in the final sequencing analysis. Larger samples can be obtained if heterozygote females can also be sequenced.
Chapter 11

Putative genetic and environmental factors influencing Attention-Deficit/Hyperactivity Disorder (ADHD) – a synthesis of the findings

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This chapter is the conclusion chapter in the thesis and discusses the findings in each chapter of the thesis in synthesis with the findings in all other chapters. The research conducted and the writing of this chapter was done by Nadia Fouché.

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Abstract

Attention-Deficit/Hyperactivity Disorder (ADHD) is a prevalent childhood onset disorder. Although research into the aetiology of ADHD abounds, findings regarding the molecular genetic architecture of the disorder have been conflicting. In this thesis, the researcher examined the plausibility of reasons for the conflicting findings in samples from South Africa. After confirming the plausibility of a genetic component to ADHD in South Africa through a familial aggregation study, the heterogenous nature of the disorder was investigated. This was done through looking at possible aetiological distinctions that can be made between different subtypes of the disorder, between male and female ADHD, between ADHD comorbid with Oppositional Defiant Disorder (ODD) and ADHD without this comorbidity, and between simplex and multiplex ADHD. Findings provided evidence for aetiological differences in ADHD between males and females, and between simplex and multiplex ADHD. Moreover, two molecular genetic studies were conducted, both examining possible reasons for the conflicting findings. The first examined the influence of gene-environment interactions in ADHD and found a significant interaction effect between a polymorphism in the dopamine transporter gene and pregnancy and/or delivery complications on ADHD symptoms. The second study examined the possibility of rare genetic variants influencing ADHD in a sample of diagnosed individuals. This study found a rare mutation in the tail-end of the fourth repeat of a 30 base pair variable number of tandem repeats polymorphism in the monoamine oxidase A gene promoter region in two siblings diagnosed with ADHD. Apart from providing tools for future researchers studying the aetiology of ADHD to reduce the conflicting findings, this thesis was also the first of its kind in South Africa and provides a basis for future research into the genetic and environmental aetiology of ADHD in the South African population.

Keywords Attention-Deficit/Hyperactivity Disorder, ADHD subtypes, Conflicting findings, Gene-environment interactions, Sex differences, Simplex and multiplex ADHD
11.1 Introduction

The aim with this thesis was to investigate factors that may contribute to the conflicting findings commonly observed for studies into the molecular genetic aetiology of Attention-Deficit/Hyperactivity Disorder (ADHD). The main objective was to be reached through investigating and answering the following research questions:

- Is there familial aggregation of ADHD symptoms in families in a South African sample, and can it thus be viewed as a heritable disorder?
- Are ADHD combined type and ADHD inattentive type distinct disorders, or varying presentations of the same disorder?
- What is the aetiological nature of the co-occurrence of ADHD and ODD in a sample from South Africa?
- What is the aetiological nature of the gender differences observed for ADHD in a sample from South Africa?
- What is the aetiological nature of simplex versus multiplex ADHD in a sample from South Africa?
- Do rare genetic variants play a role in ADHD for some patients in a sample from South Africa?
- Are there any significant interaction effects between genes and environmental factors in the aetiology of ADHD in a sample from South Africa?

The results of the studies investigating each of these research questions will be discussed in this chapter and brought into context.

11.2 Results and discussion

Chapter 4 used a family study design to determine whether the ADHD symptom dimensions of inattention, hyperactivity/impulsivity, and total ADHD aggregate in families. Aggregation in families would indicate that these symptom domains are possibly influenced by genetic factors and would provide a rationale for using genetically informative samples to answer the research questions in the rest of the thesis. More specifically, clustering of symptoms in families would indicate the influence of either additive genetic factors or the shared environment. Non-additive genetic factors would not result in similarities between first degree relatives. The results showed that inattention symptoms and total ADHD
symptoms tend to cluster in families, but not hyperactivity-impulsivity symptoms. Since total ADHD is a composite of inattention and hyperactivity-impulsivity symptoms, it can be assumed that the significant clustering found for total ADHD symptoms was due to the clustering of inattention symptoms. Therefore, it could be concluded that inattention symptoms are influenced by shared environmental factors and/or additive genetic factors in this South African sample. The lack of familial aggregation for the hyperactive-impulsive symptom dimension does not, however, mean that genetic factors do not play a role, since non-additive genetic factors could still be at play.

Evidence for genetic factors influencing ADHD in South Africa exist (Chapter 4), therefore, the research focus shifted to try to identify different subgroups of patients within the ADHD population. The existence of such subgroups will explain the conflicting findings found in molecular genetic studies of ADHD. Different subgroups of patients, if not identified prior to conducting research into the disorder, would lead to heterogeneous samples, which could result in conflicting findings. In chapter 5, the possible aetiological distinction between the ADHD combined and predominantly inattentive subtypes were explored. The hypothesis was that should the two subtypes be distinct disorders, they would “breed true” in families. In other words, relatives of individuals diagnosed with one subtype would be expected to also have that subtype. Conversely, the disorders would be seen as being variations of the same disorder if the subtypes did not “breed true” in families. The results showed that the subtypes did not “breed true” – siblings of individuals diagnosed with ADHD combined type were not significantly more likely to also have ADHD combined type, and the same for ADHD inattentive type. Therefore, the conclusion was drawn that ADHD combined and predominantly inattentive subtypes do not represent distinct disorders, but rather varying presentations of the same disorder. The conflicting findings are therefore unlikely to be due to researchers failing to distinguish between ADHD combined and ADHD inattentive type in samples of diagnosed individuals.

In chapter 6, the search for distinct subgroups continued by looking at the aetiological nature of the comorbidity between ADHD and ODD. Four hypotheses were tested, namely whether ADHD and ODD co-occur simply due to chance, whether ADHD and ODD represent an aetiologically distinct disorder from either one occurring alone, whether ADHD comorbid
Conclusions

with ODD represents a more severe variant of ADHD, and whether ADHD and ODD co-occur due to sharing causative environmental factors. Results from a multinomial logistic regression provided evidence for shared environmental factors influencing both disorders, and as a result driving their frequently reported co-occurrence. Apart from providing insight into the co-occurrence of ADHD and ODD, this finding also lends credence to the finding in chapter 4 that shared environmental factors probably have a role to play in ADHD. In addition, ADHD and ODD co-occurring due to shared environmental factors refutes the hypothesis that ADHD comorbid with ODD is a more severe form of ADHD, or that ADHD comorbid with ODD represents a distinct disorder. This negates the necessity for researchers to parse samples of diagnosed individuals into subgroups based on their ODD status, since ADHD comorbid with ODD does not represent a distinct disorder, nor a variation of the same disorder, with distinct aetiological factors. The conflicting findings in molecular genetic studies of ADHD is therefore unlikely to be due to researchers not identifying subgroups in ADHD diagnosed samples based on comorbidity with ODD.

A further factor possibly contributing to the heterogenous nature of the disorder, and consequently the mixed findings, may be differences between the sexes. ADHD has consistently been found to be more prevalent in males than in females, with males being two to three times more likely to be diagnosed with the disorder than females. In chapter 7, two models possibly explaining the sex differences found for ADHD were tested. The polygenic multiple threshold model (PMT) posits that multiple genetic and/or environmental factors influence a disorder in an additive fashion. These factors add up to make up the liability for the disorder for a particular individual. Due to having varying exposures to genetic and environmental risk factors, individuals in the population will differ in their disorder liabilities, resulting in a distribution of liability in the population. A certain critical threshold of liability needs to be overcome for the disorder to manifest in an individual. The PMT model is used to explain the differences in prevalence rates between males and females by positing that different subgroups in the population will have different critical thresholds that need to be surpassed before the disorder can manifest. In the case of ADHD, the PMT posits that females have a higher critical threshold that needs to be surpassed before the disorder will manifest compared to males. Thus, females require a greater liability than males for the disorder to occur. The second model, the mean difference model (MDM), also proposes that
a critical threshold of liability needs to be overcome prior to the disorder manifesting. However, in contrast to the PMT model, the MDM model asserts that the distribution of liability for females is shifted in the less affected direction compared to that of males, with the mean for males falling closer to the diagnostic threshold. Therefore, females do not require a greater liability than males prior to the disorder manifesting. Since the PMT model proposes that females need to carry a greater liability, including genetic liability, for the disorder to manifest, it would be expected that relatives of diagnosed females will have more severe symptoms than relatives of diagnosed males, should the PMT model prove true. Contrary to this, should the MDM model prove true, females would not need to carry a greater liability, including genetic liability, than males, and thus relatives of diagnosed females would not be expected to display more severe ADHD symptoms than relatives of diagnosed males.

The results in this study provided evidence for the validity of the PMT model, and against that of the MDM model. Individuals in families where at least one female was diagnosed with ADHD displayed greater symptom severity scores than individuals from families where only males were diagnosed with the disorder, supporting the PMT model. From these results it seems that there are aetiological differences between male and female ADHD, although these differences are more quantitative than qualitative in nature. This chapter was therefore the first in the thesis to identify a meaningful distinction between subgroups of ADHD patients. Taking gender into account in future studies may therefore lead to more aetiologically homogeneous samples, and could result in a reduction in the conflicting findings.

In chapter 8, the heterogenous nature of ADHD was further explored through trying to determine whether the disorder can be parsed into simplex and multiplex forms, with different aetiological factors influencing each. Simplex families refer to families with only one affected family member, whereas in multiplex families, at least two family members are affected by the disorder. In research on Autism Spectrum Disorders, the simplex/multiplex distinction proved to be valid, with the simplex form of the disorder mainly influenced by factors (genetic and environmental) unique to the individual, whereas the multiplex form was influenced by genetic and environmental factors shared between family members. The
simplex-multiplex distinction, if applicable, would therefore enable researchers to parse samples into subgroups where the disorder is primarily influenced by individual-specific genetic and/or environmental factors (in samples with only members from simplex families), or polygenic and/or shared environmental factors (in samples with only members from multiplex families).

The aim in chapter 8 was to determine whether the simplex-multiplex distinction was meaningful in the context of ADHD in a South African sample. This was done by testing whether environmental factors that have frequently been found to influence ADHD, namely pregnancy and delivery complications, influenced inattention and hyperactivity-impulsivity symptom severity differently in simplex versus multiplex ADHD families. The delivery complications in this study were all found to be non-shared in nature, whilst the pregnancy complications were found to be both shared and non-shared in nature. Testing main effects of pregnancy and delivery complications on inattention and hyperactivity-impulsivity symptoms showed a significant main effect for the non-shared delivery complications on both inattention and hyperactivity-impulsivity symptom severity. However, no significant main effect could be found on either of these symptom dimensions for pregnancy complications. The presence of delivery complications resulted in higher inattention and hyperactivity-impulsivity symptom severity scores.

Having found a significant main effect for non-shared delivery complications on inattention and hyperactivity-impulsivity symptom severity, the study explored whether this effect was different in simplex versus multiplex ADHD families. A significant difference was found, but only for the inattention symptom domain. Non-shared delivery complications did not influence hyperactivity-impulsivity symptom severity scores differently in simplex families compared to multiplex families. That said, a significant main effect was found for the simplex/multiplex distinction on hyperactivity-impulsivity symptom severity, with individuals from multiplex families showing greater symptom severity scores than individuals from simplex families. This finding was the first in this chapter to lend credence to the simplex/multiplex distinction in ADHD. If multiplex ADHD is indeed distinct from simplex ADHD, and influenced by factors shared between family members, all family members would be expected to show symptoms of the disorder. This would elevate the mean scores of
individuals from multiplex ADHD families when compared to individuals from simplex ADHD families, in which symptoms are influenced only by factors unique to the affected individuals, with no effect on other family members.

For inattention, the effect of non-shared delivery complications on symptom severity was restricted to simplex families. Thus, children born with non-shared delivery complications had more severe inattention symptoms, but only if they were from a simplex ADHD family. In families with more than one child diagnosed with ADHD (multiplex ADHD), non-shared delivery complications had no significant effect on inattention symptom severity. This finding was the second in this chapter to provide evidence for the validity of the simplex-multiplex distinction in ADHD, with environmental factors not shared between family members only impacting inattention symptom severity in simplex families. It might therefore be worthwhile for researchers to distinguish between simplex and multiplex forms of ADHD to reduce sample heterogeneity and consequently the conflicting findings.

In chapter 9, the focus shifted away from trying to identify different subgroups of patients within the ADHD population to determining whether gene-environment interactions play a role in ADHD. If environmental factors interact with genetic factors to influence a disorder, conflicting findings may result from molecular genetic studies not taking the applicable environmental factors into account. This is due to the environmental factors inadvertently being present for the samples in some studies, but not in others. This will result in positive associations between that genetic factor and ADHD in the studies where the intervening environmental factor is present, but not in studies where the environmental factor is absent. One of the polymorphisms that has been studied extensively in relation to ADHD, and for which results have been greatly conflicting, is a variable number of tandem repeats polymorphism in the 3’ untranslated region of the dopamine transporter gene (DAT1 3’ uVNTR). The presence of a possible interaction effect between this polymorphism and the presence of pregnancy and/or delivery complications was explored. Due to the myriad of research already conducted on maternal substance use, pregnancy and delivery complications other than maternal substance use was the focus of this study. Results showed a significant interaction effect between the presence of pregnancy and/or delivery complications and the DAT1 3’ uVNTR on both inattention and hyperactivity-impulsivity
symptom severity. The presence of pregnancy and/or delivery complications resulted in greater inattention and hyperactivity-impulsivity symptom severity, but only for individuals homozygous for the 10-repeat allele. This result may serve as a possible explanation for the mixed findings and underlines the importance for researchers to take environmental factors, along with genetic factors, into account when studying the aetiology of ADHD.

The findings in chapters 8 and 9 underlines the complex nature of the factors influencing ADHD. Although findings from the two chapters are not directly comparable since a composite index of pregnancy and/or delivery complications was used in chapter 9, but the factors were considered separately in chapter 8, the findings in these two chapters do show the importance of considering moderating variables when looking at the impact of environmental factors on ADHD. Making use of multiple moderating variables in the same study may be particularly informative, and, given the findings in this thesis, future research may consider moderating for both simplex/multiplex ADHD and the DAT1 3’ uVNTR polymorphism when considering the impact of environmental factors on ADHD. In addition, given the findings of the divergence between the genders in chapter 7, running this multiple moderator analysis separately for males and females may also prove worthwhile.

Chapter 10 was the second chapter focusing on the molecular genetic architecture of ADHD. The hypothesis was that the mixed findings found thus far in molecular genetic studies of the disorder may be due to rare genetic variants playing a role in ADHD for a subgroup of patients. Two contrasting hypotheses came into play in this chapter. The common disease/common variant (CDCV) hypothesis postulates that high frequency diseases in the human population are due to common genetic variations. In contrast, the common disease/rare variant (CDRV) hypothesis asserts that a multitude of rare, mildly deleterious genetic variations with relatively high penetrance are major underlying factors in common human disease. Focusing only on common genetic variants in samples which include individuals carrying rare genetic variants that influence the disorder may result in a lack of positive association between a particular candidate polymorphism and ADHD. This may consequently contribute to the mixed findings.

One form of rare genetic variant for which research is lacking is rare DNA sequence variations within repeat motifs of variable number of tandem repeat (VNTR) polymorphisms.
The polymorphism focused on in this chapter is a variable number of tandem repeats polymorphism in the promoter region of the monoamine oxidase A gene (MAOA-uVNTR). This polymorphism has been associated with ADHD in some studies, whilst other studies failed to find any positive association. The MAOA-uVNTR consists of a 30 base pair sequence that is repeated a variable number of times. In line with the common disease/rare variant hypothesis, the aim of this chapter was to scrutinise this polymorphism in children diagnosed with ADHD to determine whether any rare mutations of the 30 base pair sequence could be detected. The majority of the sample carried only the commonly observed wild-type 30 base pair sequence, lending credence to the common disease/common variant hypothesis. However, a rare mutation of the 30 base pair sequence was observed in two siblings in the sample. These individuals both carried a rare deletion of 12 base pairs at the tail end of the fourth repeat of the 30 base pair sequence. This mutation has never before been described in either the molecular genetic literature related to ADHD, or the human molecular genetic literature at large. Although more research is needed to determine the prevalence of this mutation in world populations, and also to determine whether this rare mutation is associated with ADHD in some cases, this finding highlights the importance for future research to not only focus on the number of repeats present in VNTR’s, but to also examine sequence variations within the repeat sequences.

Taken together, the research undertaken in this thesis resulted in a number of important findings which can be used by future researchers to reduce the conflicting findings in molecular genetic studies of ADHD. In addition, statistical and methodological tools were tested which can be used by future researchers in behavioural genetics in South Africa. This study provided evidence that additive genetic factors and/or shared environmental factors play a role in the inattention symptom dimension of ADHD. Future research should examine the symptoms dimensions of inattention and hyperactivity-impulsivity separately when investigating genetic and environmental factors influencing the disorder. The influence of the shared environment was again emphasised in the study on the co-occurrence of ADHD and ODD, negating initial findings that the influence of the shared environment on ADHD is negligible. Researchers in future may therefore want to consider the shared environment as a possible factor when designing and conducting studies into the aetiology of ADHD, rather than assuming that it has no influence. Furthermore, the finding that ADHD and ODD
frequently co-occur due to shared environmental factors refutes the possibility that ADHD comorbid with ODD is a distinct disorder from ADHD occurring alone. Since the aetiology should therefore not differ between ADHD and ADHD comorbid with ODD, there is no need to parse samples into ADHD only and ADHD plus ODD prior to conducting research into the aetiology of ADHD.

In contrast, the finding supporting the PMT model for explaining the gender differences in ADHD shows that it might be worthwhile for researchers to distinguish between males and females when conducting research into the aetiology of the disorder. This finding pointed to males and females differing quantitatively, rather than qualitatively, in the aetiological factors influencing ADHD. Further evidence for the heterogeneous nature of the disorder was found in the study showing that pregnancy and/or delivery complications influenced inattention symptom severity, but only in members from families where only one child was diagnosed with ADHD (simplex ADHD). Conflicting findings may therefore be reduced in future research if researchers distinguish between simplex and multiplex ADHD prior to conducting research into the aetiology of ADHD. A further reduction in conflicting findings may result if environmental factors are taken into account when studying the molecular genetic aetiology of ADHD. This was shown through the significant interaction effect that was found between pregnancy and/or delivery complications and the DAT1 3’ uVNTR on ADHD symptoms. Also, rare genetic variants may also have a role to play for a subgroup of patients with the disorder, as was shown through the rare mutation in the MAOA-uVNTR that was identified for two siblings diagnosed with ADHD. Finally, given the significant findings for moderating variables when considered separately in this study, considering multiple moderator variables at the same time when conducting studies on genetic and environmental factors influencing ADHD may be of particular value for future research aiming to explain and reduce the conflicting findings.

11.3 Conclusion

The findings of this thesis provided evidence that the conflicting findings found thus far for the molecular genetic aetiology of ADHD may be due to the heterogeneous nature of the disorder, and researchers not taking this into account when selecting samples. More homogeneous samples, and consequently less conflicting findings, may result if researchers
take gender, simplex and multiplex ADHD, gene-environment interactions, and rare genetic variants into account when researching the aetiology of ADHD. Moreover, this was the first study of its kind in a South African sample to use statistical and molecular genetic tools to comprehensively examine the genetic and environmental aetiology of ADHD, and to find possible reasons for the mixed findings. This study may therefore serve as a basis and starting point for future research into the genetic architecture of ADHD in South Africa.

11.4 Limitations of the study

A number of important limitations to this study should be noted. Cost and time constraints limited the sample size, especially for the molecular genetic component of the thesis. The findings should be replicated using larger samples. In addition, a non-probability sampling strategy was utilised, resulting in a sample that was not representative of the South African population at large. This is common in behavioural genetic studies published in peer-reviewed journals, but nonetheless precludes the generalization of findings from the sample to the South African population. Future studies into the behavioural genetics of ADHD in South Africa should attempt to obtain representative samples, but can still make use of the statistical tools and family-study design methodology explored in this study.

A further limitation was due to the decision to make use of parent-report of ADHD diagnosis by a healthcare professional for the statistical genetic chapters in this thesis, rather than having all participants in the sample directly diagnosed by a healthcare professional. This decision was made due to the budget and time allocated for the study not allowing all participants to undergo a formal structured diagnostic interview directly by a healthcare provider, and also after noting that studies making use of parent-report of ADHD diagnosis by a healthcare provider have been published successfully in peer-reviewed journals. Should time and budget allow, future studies may consider rather making use of formal structured diagnostic interviews by healthcare providers to classify all participants as diagnosed with ADHD or not.

Due to this being the first study of its kind to be conducted in a South African sample, exclusion criteria for the sample were relaxed. More stringent exclusion criteria would be preferable in future studies on the behavioural genetics of ADHD in South Africa. Finally,
since the main focus of this thesis was on exploring genetic factors through either statistical or molecular genetic techniques, it was decided to limit the length of the questionnaires that needed to be completed, and thereby encourage participation, through not including the myriad of possible confounding variables in this study. It may be informative, however, to take factors such as psychiatric comorbidity, intelligence, education, and socio-economic status amongst others, into account when conducting similar studies in South Africa in the future.
Chapter 12

Summary and Opsomming

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Attention-Deficit/Hyperactivity Disorder (ADHD) is a prevalent childhood onset externalising disorder for which the negative ramifications have been well documented. Although the disorder has been shown to be highly heritable, molecular genetic studies into the aetiology of the disorder have been plagued by conflicting findings. The aim of this thesis was to find reasons for the mixed findings, focusing particularly on the heterogeneous nature of the disorder. This was done to provide future researchers with tools that would enable them to not only select more homogeneous samples, but to also take pertinent confounding factors into account when studying the aetiology of ADHD. The ultimate aim was to reduce the conflicting findings regarding the aetiology of ADHD, and in doing so enabling a better understanding of the disorder to be gained. In addition, this study aimed to provide information on genetic and environmental factors influencing ADHD in a South African sample, a country where there is a dearth of knowledge in this regard.

The possibility of genetic factors playing a role in ADHD in South Africa was first confirmed through a family study examining whether ADHD symptoms aggregate in families. Results showed that inattention and total ADHD symptoms do aggregate in families, and is therefore influenced by additive genetic and/or shared environmental factors. In subsequent chapters, reasons for the mixed findings were tested. Findings showed that neither the ADHD subtypes, nor ADHD comorbid with ODD, represent distinct disorders. Rather, the subtypes were found to be variations of the same underlying disorder, whilst the frequent co-occurrence of ADHD and ODD was found to be due to shared environmental factors influencing both. The study did, however, show aetiological differences in ADHD between males and females, although these differences were more quantitative than qualitative in nature. Females required a greater liability of risk factors before manifesting the disorder compared to males. Moreover, delivery complications were found to influence ADHD symptoms in families where only one
child was diagnosed with the disorder (simplex ADHD), but not in families with more than one diagnosed family member (multiplex ADHD).

Finally, two molecular genetic studies were conducted to determine the effect of gene-environment interactions on ADHD, as well as examine the possibility that rare genetic variants influence the disorder. The first study showed a significant interaction effect between pregnancy and/or delivery complications and a variable number of tandem repeats polymorphism in the dopamine transporter gene (DAT1 3’ uVNTR) on ADHD symptom severity. Pregnancy and/or delivery complications played a role in ADHD symptom severity, but only in individuals homozygous for the 10-repeat allele of the DAT1 3’ uVNTR. This result points to the DAT1 3’ uVNTR having an indirect rather than a direct effect on ADHD symptom severity, and serves as a possible explanation for the mixed findings. The second molecular genetic study tested whether any rare genetic variants of the monoamine oxidase A variable number of tandem repeats polymorphism (MAOA-uVNTR) could be found in a sample of children diagnosed with ADHD. Results from this study showed a rare deletion of 12 base pairs at the tail-end of the fourth repeat of the 30 base pair sequence in two siblings diagnosed with ADHD.

In conclusion, findings showed that a reduction in conflicting findings may result if researchers distinguish between the sexes, as well as between simplex and multiplex ADHD, prior to conducting research into the aetiology of the disorder. Moreover, a further reduction in the conflicting findings may result if researchers take gene-environment interactions into account when studying genetic and environmental factors influencing ADHD. Finally, it may be worthwhile for researchers to search for rare mutations in well-known polymorphisms when studying the genetic aetiology of ADHD.

**Keywords** Additive genetic factors, Attention-Deficit/Hyperactivity Disorder, Dopamine system genes, Familial symptom aggregation, Gene-environment interactions, Hyperactivity-impulsivity, Inattention, Oppositional defiant disorder, Polygenic multiple threshold model, Pregnancy and delivery complications, Rare genetic variants, Shared environmental factors, Simplex and multiplex ADHD
Summary

Aandagtekort-hiperaktiwiteitsversteuring (ATHV) is ‘n wydverspreide eksternaliseringsversteuring wat begin in die kinderjare, en waarvoor die negatiewe gevolge wel bekend is. Alhoewel daar bewyse is dat die versteuring hoogs oorerflik is, het mollekulêre studies tot dusver kontrasterende resultate gelewer. Die doel van die tesis was om redes te vind vir hierdie kontrasterende resultate, veral deur te fokus op die heterogene aard van die steurnis. Dit is gedoen om toekomstige navorsers met die nodige hulpmiddels te voorsien wat hulle in staat sal stel om nie net meer homogene steekproewe te trek nie, maar ook om pertinente steuringsveranderlikes in ag te neem wanneer die etiologies van die versteuring nagevors word. Die uiteindelike doel was om ‘n afname in die kontrasterende resultate rakende die etiologie van ATHV te bewerkstellig, en sodoende ‘n beter begrip van die versteuring te bevorder. Daarbenewens het die studie ten doel gehad om inligting te verskaf oor die genetiese en omgewingsfaktore wat ‘n rol speel in ATHV in Suid Afrikaanse steekproef, ‘n land waar daar ‘n tekort aan inligting in hierdie verband is.

Die moontlikheid dat genetiese faktore ‘n rol speel in ATHV in Suid Afrika is eers bevestig deur die doen van ‘n familie studie waarin getoets is of ATHV simptome ooreenstem tussen familielede. Resultate het getoon dat aandagtekort simptome, asook totale ATHV simptome, wel ooreenstem tussen familielede, en dus beïnvloed word deur byvoegende genetiese faktore en/of gedeelde omgewingsfaktore. In die daaropvolgende hoofstukke is redes vir die kontrasterende resultate getoets. Bevindinge het getoon dat nie die ATHV subtipes, of ATHV komorbid met Opposisionele-Uitdaging Versteuring (OUV), gesien kan word as unieke versteurings nie. Daar is eerder bevind dat die subtipes variasies van dieselfde onderliggende versteuring is, terwyl die dikwelse komorbiditeit tussen ATHV en OUV toegeskryf kan word aan gedeelde omgewingsfaktore wat beide versteurings beïnvloed. Die studie het egter etiologiese verskille getoon tussen ATHV in mans en in vrouens, alhoewel hierdie verskille meer kwantitatief as kwalitatief van aard was. Vrouens benodig ‘n groter
las van risiko faktore voor die versteuring manifesteer in vergelyking met mans. Verder is geboortekomplikasies gevind om ATHV simptome te beïnvloed in families waar slegs een kind met ATHV gediagnoseer is (simplistiese ATHV), maar nie in families waar meer as een kind met die versteuring gediagnoseer is nie (komplekse ATHV).

Uiteindelik, is twee mollekulêr genetiese studies uitgevoer om die invloed van geen-omgewinginteraksies op ATHV te bepaal, asook om die moontlikheid te ondersoek dat skaars genetiese variantie die versteuring beïnvloed. Die eerste studie het ‘n betekenisvolle interaksie effek getoon tussen swangerskap en/of geboorte kompleksies en ‘n basispaar herhalingspolimorfisme in die dopamien transportergeen (DAT1 3’ uVNTR), op die erns van ATHV simptome. Swangerskap en/of geboorte kompleksies het ‘n rol gespeel in die erns van ATHV simptome, maar slegs in individue wat homosigoties is vir die 10-herhaling alleel van die DAT1 3’ uVNTR. Hierdie resultate dui daarop dat die DAT1 3’ uVNTR ‘n indirekte, eerder as ‘n direkte effek op die erns van ATHV simptome het, en dien as ‘n moontlike verduideliking vir die kontrasterende resultate. Die tweede mollekulêr genetiese studie het getoets of enige skaars genetiese variantie binne die Momoamien Oksidase A basispaar herhalingspolimorfisme (MAOA-uVNTR) in ‘n steekproef van kinders gediagnoseer met ATHV gevind kon word. Resultate van die studie het ‘n skaars delesie van 12 basispare aan die stertkant van die vierde herhaling van die 30 basispaar formasie getoon in twee sibbe wat met ATHV gediagnoseer is.

Ten slotte het die resultate van die studie getoon dat ‘n afname in kontrasterende bevindinge kan volg indien navorsers onderskeiding tref tussen die geslagte, asook tussen simplistiese en komplekse ATHV, wanneer navorsing rakende die etiologie van ATHV uitgevoer word. Verder mag ‘n verdere afname in kontrasterende resultate volg indien navorsers geen-omgewing interaksies in ag neem wanneer die genetiese en omgewingsfaktore wat ATHV beïnvloed nagevors word. Einde ten laaste, mag dit ook die moeitewerd wees vir navorsers om te soek vir skaars mutaties binne welbekende polimorfismes wanneer die genetiese etiologie van ATHV nagevors word.
**Sleutelwoorde** Aandagtekort-hiperaktiwiteitsversteuring, Aandagtekort, Byvoegende genetiese faktore, Dopamiensisteem gene, Gedeelde omgewingsfaktore, Geen-omgewing interaksies, Hiperaktiwiteit en impulsiwiteit, Opposisionele-uitdaging versteuring, Poligeniese meervoudige drumpelmodel, Simplistiese en komplekse ATHV, Simptoom ooreenstemming binne families, Skaars genetiese variante, Swangerskap en geboorte komplikasies
Chapter 13

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APPENDICES

Appendix A: Information leaflets and informed consent forms
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Appendix F: Letter for ethical approval of the study
Appendix A: Information leaflets and informed consent forms

Appendix A1: Information leaflet given to participants to read so that they could provide informed consent to participate in the study

PROJECT TITLE: Putative genetic and environmental factors influencing Attention-Deficit/Hyperactivity Disorder (ADHD) in a South African sample.

Dear participant

We, the Genetics Department at the University of the Free State, are doing research on the variable influence of genetic and environmental factors on Attention-Deficit/Hyperactivity Disorder (ADHD) symptom severity in South African children. Research is just the process to learn the answer to a question. Genetic factors refer to biological units that are passed on from parents to their children and, among other things influence how people look and behave. Environmental factors in this sense refer to any factors in a child’s environment other than genetic factors that may have an influence on their ADHD symptoms. In this study we want to learn whether certain genes influence the severity of ADHD symptoms, and also whether environmental factors surrounding pregnancy and birth influence ADHD symptom severity. Participating in this study may not directly benefit you or your children. However, identifying the role of genetic and environmental factors in ADHD will lead to a better understanding of factors causing or influencing this disorder, and may aid not only in the diagnoses of the disorder, but also in its management. Earlier diagnoses and better management may improve not only your child’s life, but also that of your family.

We are inviting you to participate in this research study, and also asking for your permission to include your child in this research study. You participation will entail the once-off completion of a set of questionnaires for yourself and your children. In addition, should you consent, a saliva sample will be taken from each of your children by asking them to spit into a small tube for the purposes of genetic analyses. This should not take more than 30 minutes of your time, and only five minutes of your children’s time. You are not required to provide any identifying particulars on the questionnaires, although sets of questionnaires from parents will be matched with that of their children by means of a numbering system. This same numbering system will also be used to match the saliva samples to a particular family. Consequently, you and your children will remain anonymous. Furthermore, both the genetic information and information gathered in the questionnaires will be treated with the strictest confidence. The genetic information will ONLY be used for the purposes of this study, and due to the numbering system will not be linkable to your child in any way. The results of this study may, however, be published in a scientific journal, but in this instance you will also remain anonymous, although the cohort of participants may be identified (e.g. people reading the journal article may see that the sample consisted of South African children and their parents).
Also, the company responsible for the data analysis, Melody M Consulting, will see the data, but you will still remain anonymous.

It is not foreseen that your physical or emotional wellbeing, or that of your children, will be adversely affected by participating in this study. However, your participation in this study is completely voluntary, and you are free to withdraw from the study at any time and doing so will not hold any consequences for yourself or your children. You will not incur any expenses as a result of your participation in this study. Similarly you will receive no compensation for your participation in the study. However, should there be any unforeseen “out of pocket” expenses for you, you will be reimbursed.

Due to us not asking for any identifying particulars, we will not be able to provide participants with the results of the study.

Please feel free to contact myself or Prof Johan Spies (University of the Free State) with any questions you may have regarding this study, or should you want to report any study-related adverse events. You may also report any complaints or problems to the Secretariat of the Ethics Committee of the Faculty of Health Sciences, University of the Free State, by contacting them at the following telephone number: (051) 4052812.

Thank you for your cooperation

Researcher: Nadia Laubscher (0824124959) (laubschernadia2@gmail.com)

Supervisor: Prof J.J. Spies (johanspies77@gmail.com)
Appendix A2: Informed consent form for participants to sign to show that they willingly agreed to participate in the research

You have been asked to participate in a research study, and also to consent to your children participating in the study. You have been informed about the study by Miss Nadia Laubscher. You may contact Nadia Laubscher at 0824124959 any time you have questions about the research. You may contact the Secretariat of the Ethics Committee of the Faculty of Health Sciences, UFS at telephone number (051) 4052812 if you have questions about your or your child’s rights as a research subject. Your participation, as well as that of your child/children, in this research is voluntary, and neither you nor your child/children will be penalized or lose benefits if you refuse to participate or decide to terminate participation. If you agree to participate, you will be given a signed copy of this document as well as the participant information sheet, which is a written summary of the research. Your child/children will also be given age-appropriate information sheets. Due to the genetic nature of the study, a separate information sheet and consent form will also be made available to you containing information on the storing of samples and use for future research.

The research study, including the above information is clear to me. I understand what my involvement in the study means, as well as what the involvement of my child/children mean, and I voluntarily agree to participate, and also consent to my child/children’s participation.

__________________________  __________________________
Signature of Participant                  Date
Appendix A3: Information document regarding genetics research given to participants to read so that they could provide informed consent to participate in the molecular genetic aspect of the study

Some information on the study

We are planning a research project on the variable influence of genetic and environmental factors on Attention-Deficit/Hyperactivity Disorder (ADHD) symptom severity in South African children, and request your permission to use some of your children’s tissue (specifically, saliva) for laboratory tests. The tests will involve an analysis of the genetic composition of saliva samples, and are aimed at increasing the understanding of the causes and behaviour of your child’s/children’s condition. Genes are what you inherit from your parents. They are found in every part of the body and therefore they will be present in your child’s saliva which we will obtain. The findings of this study will not have direct bearing on the management of your child’s disorder. The findings may, however, eventually benefit others in terms of diagnoses and treatment of the condition. You are free to refuse consent and you do not have to give reasons for doing so.

Privacy and Confidentiality

The following arrangements have been made to ensure privacy and confidentiality of your child/children’s genetic information:

- We will make use of a numbering system to identify samples, and thus no names or any other identifying particulars will be written on the samples.
- Consequently, we will not know who the DNA we analyse belong to.
- Each family that participates will be given a number, starting from the number one. Each child within the family will, according to their order of birth, get an additional number to the number one. Thus, the first child of family one will receive the number 101, and the second child of family two will receive the number 202.
- In this way, siblings will be linked, but in a way where anonymity is ensured.
- All questionnaires will be kept in a locked cabinet in the Department of Genetics, and no one apart from the researchers will have access to it.
- All genetic material will also be kept in locked fridges in the Department of Genetics, and will only be accessible by the researchers.

Your child/children’s genetic material and information will thus be used in an unidentifiable form. Because your child/children’s material or information is to be made unidentifiable, it will not be possible to provide you with personal research results. The nature of this genetic research study is such that it is highly unlikely that it will reveal information of potential importance to the future health of a participant or the participants’ offspring. This is because behavioural disorders are influenced by both genes and the environment, and thus a single variation in a gene, or even a number of genes, will not be sufficient to cause the disorder.

Results of research
Researchers will endeavour to provide information about the outcome of the research in the form of a popular media article. It is not intended to provide direct feedback to participants because of the anonymous nature of the study.

**Family members**

Information from other family members apart from your children will not be required for this research. Thus, none of your other family members will be approached in any way. This research does not have the potential to detect non-paternity or non-maternity, since we are only obtaining samples from your children, and not you as their parents. Your child/children’s material and information will not be released for other uses without consent, unless required by law.

**Storage**

We would like to retain some of the same tissue in storage for possible future research related to the present research question. The duration of storage will be a maximum of 10 years. If you are unhappy to have your child/children’s tissue stored for future research, their genetic material and information will be disposed of at the end of the study, once the sample storage and record-keeping requirements of good research practice have been met. All genetic material will be stored in a locked -80 degree Celsius fridge in the laboratories of the Department of Genetics.

Please select one of the options below regarding the storing of your child’s genetic material after the current study:

- a) I do not want to have my child’s genetic material stored for future research, please dispose of the material immediately after completion of the current study
- b) I do not mind having my child’s genetic material stored for future research

If you selected option (b) above, your child’s genetic material will be disposed of immediately following the completion of the current study.

Do you have any sensitivity on how your child/children’s tissues should be disposed of? If so, what are they? (Please write your answer in the space provided below. These will be recorded and taken into account at the time of disposal.)

---

**Voluntary participation**
You do not have to agree to allow your child to take part in this research and you are free to have your child/children withdraw from the research at any time. Your child/children’s medical treatment will not be compromised in any way if they do not participate.
Appendix A4: Informed consent form for participants to sign to show that they willingly agreed to participate in genetic research

You have been asked for your consent to have your children participate in a genetic research study. You have been informed about the study by Miss Nadia Laubscher. You may contact Nadia Laubscher at 0824124959 any time you have questions about the research. You may contact the Secretariat of the Ethics Committee of the Faculty of Health Sciences, UFS at telephone number (051) 4052812 if you have questions about your child/children’s rights as a research subject. Your child/children’s participation in this research is voluntary, and neither you nor they will be penalized or lose benefits if you refuse to have them participate or decide to terminate their participation. If you agree for them to participate, you will be given a signed copy of this document as well as the participant information sheet, which is a written summary of the research.

The research study, including the above information has been verbally described to me. I understand what my child/children’s involvement in the study means and I voluntarily agree to allow them to participate.

______________________________  ______________________
Signature of Participant          Date
Appendix B: Questionnaires

Appendix B1: Self-compiled biographical questionnaire given to parents to complete for the proband and all of his/her siblings as a means to gather biographical information, as well as information related to ADHD diagnosis and pregnancy and delivery complications.

Please complete the next set of questions by marking the appropriate choice with an X in the open block to the RIGHT of the option you choose, or writing the answer in the space provided.

<table>
<thead>
<tr>
<th>1</th>
<th>Questions about the child for whom you are completing the questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>General:</td>
</tr>
<tr>
<td>1.1.1</td>
<td>Age of child:</td>
</tr>
<tr>
<td>1.1.2</td>
<td>This child is my: (first child; second child; etc.)</td>
</tr>
<tr>
<td>1.1.3</td>
<td>Child’s gender: Male Female</td>
</tr>
<tr>
<td>1.1.4</td>
<td>Child’s current school and grade:</td>
</tr>
<tr>
<td>1.1.4</td>
<td>For the mother: Are you this child's biological mother? Yes No</td>
</tr>
<tr>
<td>1.1.5</td>
<td>For the father: Are you this child's biological father? Yes No</td>
</tr>
<tr>
<td>1.1.6</td>
<td>Has this child been adopted? Yes No</td>
</tr>
<tr>
<td>1.2</td>
<td>ADHD:</td>
</tr>
<tr>
<td>1.2.1</td>
<td>Has this child been formally diagnosed with ADHD? Yes No</td>
</tr>
<tr>
<td>1.2.1.1</td>
<td>If yes, who diagnosed your child? (e.g. Family doctor, paediatrician, psychologist, school psychologist)</td>
</tr>
<tr>
<td>1.2.2</td>
<td>Do you suspect that this child has ADHD (if not diagnosed)? Yes No</td>
</tr>
<tr>
<td>1.2.3</td>
<td>Is this child currently on medication for ADHD? Yes No</td>
</tr>
<tr>
<td>1.2.3.1</td>
<td>If the child is currently on medication for ADHD, please specify type:</td>
</tr>
<tr>
<td>1.2.3.2</td>
<td>Please specify the period of time that your child has been on this medication:</td>
</tr>
<tr>
<td>1.2.3.3</td>
<td>Please specify any side-effects due to the medication:</td>
</tr>
<tr>
<td>1.2.3.4</td>
<td>Do you think this medication is effective for your child's ADHD symptoms? Yes No</td>
</tr>
<tr>
<td>1.2.4</td>
<td>Has this child previously taken any other type of medication for ADHD? Yes No</td>
</tr>
<tr>
<td>1.2.4.1</td>
<td>If yes to 1.2.4, please specify type:</td>
</tr>
<tr>
<td>1.2.4.2</td>
<td>Were there any negative side effects from this medication? Yes No</td>
</tr>
</tbody>
</table>
If there were negative side effects, please specify:

| 1.2.4.3 | If yes to 1.2.4, why did you decide to stop letting your child take this medication? |

### 1.3  Pregnancy and birth

<table>
<thead>
<tr>
<th>1.3.1</th>
<th>Did you experience any medical complications during your pregnancy with this child:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

| 1.3.1.1 | If yes to 1.3.1, please specify: |

<table>
<thead>
<tr>
<th>1.3.2</th>
<th>Was this child born too early?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

| 1.3.2.1 | If yes to 1.3.2, please indicate month of pregnancy in which child was born: |

<table>
<thead>
<tr>
<th>1.3.3</th>
<th>Were there any complications during the birth of this child?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

| 1.3.3.1 | If yes to 1.3.3, please specify: |

<table>
<thead>
<tr>
<th>1.3.4</th>
<th>Did this child have a very low birth weight?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1.3.5</th>
<th>During your pregnancy with this child, did you smoke cigarettes (For the mother)?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1.3.5.1</th>
<th>If yes to 1.3.5, please indicate roughly how much you smoked:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A few times, less than 1 cigarette per day</td>
</tr>
</tbody>
</table>

| 1.3.6 | In which town/city was this child born? |

### 1.4  Medical and psychological history of child

<table>
<thead>
<tr>
<th>1.4.1</th>
<th>Has this child ever been diagnosed with a psychological disorder (apart from ADHD) (eg. Depression)?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

| 1.4.1.1 | If yes to 1.4.1, please specify: |

<table>
<thead>
<tr>
<th>1.4.2</th>
<th>Has this child ever been diagnosed with epilepsy?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1.4.3</th>
<th>Has this child ever suffered a serious head injury?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1.4.4</th>
<th>Is there any other medical disorder that this child has been diagnosed with?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

| 1.4.4.1 | If yes to 1.4.4, please specify: |
Appendix B2: SNAP-IV 26-item Teacher and Parent Rating Scale given to parents to complete for the proband and all of his/her siblings as a means to gather ADHD symptom severity scores

For each of the next set of items, please make an X in the open block to the RIGHT of the choice that best describes this child. If the child is on medication for ADHD, please select responses for both the "before medication" and "after medication" options. If this child is not on medication for ADHD, or has not been diagnosed with ADHD, please ignore the "after medication" option, and select a response only for the "before medication" option:

<table>
<thead>
<tr>
<th></th>
<th>Often fails to give close attention to details or makes careless mistakes in schoolwork or tasks:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Before medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
<tr>
<td></td>
<td>After medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often has difficulty sustaining attention in tasks or play activities:</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Before medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
<tr>
<td></td>
<td>After medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often does not seem to listen when spoken to directly:</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Before medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
<tr>
<td></td>
<td>After medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often does not follow through on instructions and fails to finish schoolwork, chores, or duties:</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Before medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
<tr>
<td></td>
<td>After medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often has difficulty organizing tasks and activities:</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Before medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
<tr>
<td></td>
<td>After medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often avoids, dislikes, or reluctantly engages in tasks requiring sustained mental effort:</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Before medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
<tr>
<td></td>
<td>After medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often loses things necessary for activities (e.g., toys, school assignments, pencils, or books):</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Before medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
<tr>
<td></td>
<td>After medication:</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td></td>
<td>Just a little</td>
</tr>
<tr>
<td></td>
<td>Quite a bit</td>
</tr>
<tr>
<td></td>
<td>Very much</td>
</tr>
<tr>
<td></td>
<td>Often is distracted by extraneous stimuli:</td>
</tr>
<tr>
<td>---</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Before medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>After medication: Not at all</td>
</tr>
<tr>
<td>9</td>
<td>Often is forgetful in daily activities:</td>
</tr>
<tr>
<td></td>
<td>Before medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>After medication: Not at all</td>
</tr>
<tr>
<td>10</td>
<td>Often has difficulty maintaining alertness, orienting to requests, or executing directions:</td>
</tr>
<tr>
<td></td>
<td>Before medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>After medication: Not at all</td>
</tr>
<tr>
<td>11</td>
<td>Often fidgets with hands or feet or squirms in seat:</td>
</tr>
<tr>
<td></td>
<td>Before medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>After medication: Not at all</td>
</tr>
<tr>
<td>12</td>
<td>Often leaves seat in classroom or in other situations in which remaining seated is expected:</td>
</tr>
<tr>
<td></td>
<td>Before medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>After medication: Not at all</td>
</tr>
<tr>
<td>13</td>
<td>Often runs about or climbs excessively in situations in which it is inappropriate:</td>
</tr>
<tr>
<td></td>
<td>Before medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>After medication: Not at all</td>
</tr>
<tr>
<td>14</td>
<td>Often has difficulty playing or engaging in leisure activities quietly:</td>
</tr>
<tr>
<td></td>
<td>Before medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>After medication: Not at all</td>
</tr>
<tr>
<td>15</td>
<td>Often is &quot;on the go&quot; or often acts as if &quot;driven by a motor&quot;:</td>
</tr>
<tr>
<td></td>
<td>Before medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>After medication: Not at all</td>
</tr>
<tr>
<td>16</td>
<td>Often talks excessively:</td>
</tr>
<tr>
<td></td>
<td>Before medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>After medication: Not at all</td>
</tr>
<tr>
<td></td>
<td>Often blurs out answers before questions have been completed:</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often has difficulty awaiting turn:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often loses temper:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often argues with adults:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often actively defies or refuses adult requests or rules:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often deliberately does things that annoy other people:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often blames others for his or her mistakes or misbehaviour:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often touchy or easily annoyed by others:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often is angry and resentful:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Often is spiteful or vindictive:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td><strong>After medication:</strong> Not at all</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Before medication:</td>
<td></td>
</tr>
<tr>
<td>After medication:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix B3: Self-compiled biographical questionnaire given to the parents to complete for themselves as a means to gather biographical information, as well as information on their medical histories

Please complete the next set of questions by marking your chosen response with an X in the open block provided to the RIGHT of each option, or by writing the answer in the space provided.

<table>
<thead>
<tr>
<th>1</th>
<th>Questions about you as the child’s parents:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>General</td>
</tr>
<tr>
<td>1.1.1</td>
<td>Age of mother:</td>
</tr>
<tr>
<td>1.1.2</td>
<td>Age of father:</td>
</tr>
<tr>
<td>1.1.3</td>
<td>Where do you currently live? Please provide name of city/town and district:</td>
</tr>
<tr>
<td>1.1.4</td>
<td>Race (for statistical purposes only):</td>
</tr>
<tr>
<td>1.1.5</td>
<td>Home language:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1.2</th>
<th>Medical histories</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2.1</td>
<td>Have you ever been diagnosed with ADHD?</td>
</tr>
<tr>
<td>1.2.1.1</td>
<td>If yes to 2.2.1, at what age were you diagnosed?</td>
</tr>
<tr>
<td>1.2.2</td>
<td>Has anyone in your family (eg. Mom, dad, grandmother, etc.) ever been diagnosed with ADHD?</td>
</tr>
<tr>
<td>1.2.2.1</td>
<td>If yes to 2.2.2, please specify relation to family member (eg. mom, dad, etc.)</td>
</tr>
<tr>
<td>1.2.3</td>
<td>Have you ever been diagnosed with another psychological disorder (apart from ADHD)?</td>
</tr>
<tr>
<td>1.2.3.1</td>
<td>If yes to 2.2.3, please specify disorder (eg. Depression):</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>For the father:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2.4</td>
</tr>
<tr>
<td>1.2.4.1</td>
</tr>
<tr>
<td>1.2.5</td>
</tr>
<tr>
<td>1.2.5.1</td>
</tr>
<tr>
<td>1.2.6</td>
</tr>
<tr>
<td>1.2.6.1</td>
</tr>
</tbody>
</table>
Appendix B4: The Adult ADHD Self-Report Scale (ASRSv1.1) Symptom Checklist given to the parents to complete for themselves as a means to gather parental ADHD symptom severity scores

Please answer the questions below, rating yourself on each of the criteria shown using the scale to the right of the questions. As you answer each question, place an X in the box that best describes how you have felt and conducted yourself over the past 6 months.

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong></td>
<td>How often do you have trouble wrapping up the final details of a project, once the challenging parts have been done?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>2</strong></td>
<td>How often do you have difficulty getting things in order when you have to do a task that requires organization?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>3</strong></td>
<td>How often do you have problems remembering appointments or obligations?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>4</strong></td>
<td>When you have a task that requires a lot of thought, how often do you avoid or delay getting started?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>5</strong></td>
<td>How often do you fidget or squirm with your hands or feet when you have to sit down for a long time?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>6</strong></td>
<td>How often do you feel overly active and compelled to do things, like you were driven by a motor?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>7</strong></td>
<td>How often do you make careless mistakes when you have to work on a boring or difficult project?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>8</strong></td>
<td>How often do you have difficulty keeping your attention when you are doing boring or repetitive work?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>9</strong></td>
<td>How often do you have difficulty concentrating on what people say to you, even when they are speaking to you directly?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>10</strong></td>
<td>How often do you misplace or have difficulty finding things at home or at work?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>11</strong></td>
<td>How often are you distracted by activity or noise around you?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>12</strong></td>
<td>How often do you leave your seat in meetings or other situations in which you are expected to remain seated?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>13</strong></td>
<td>How often do you feel restless or fidgety?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>14</strong></td>
<td>How often do you have difficulty unwinding and relaxing when you have time to yourself?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
<tr>
<td><strong>15</strong></td>
<td>How often do you find yourself talking too much when you are in social situations?</td>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
</tr>
</tbody>
</table>
16. When you're in a conversation, how often do you find yourself finishing the sentences of the people you are talking to, before they can finish them themselves?

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Very often</th>
</tr>
</thead>
</table>

17. How often do you have difficulty waiting your turn in situations when turn taking is required?

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Very often</th>
</tr>
</thead>
</table>

18. How often do you interrupt others when they are busy?

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Very often</th>
</tr>
</thead>
</table>
Appendix C: Genotyping results for chapter 9

Appendix C1: Agarose gel electrophoresis results for the polymorphism in the DAT1 gene investigated in chapter 9 (DAT1 3’ uVNTR)

*Note: Not all individuals who were successfully genotyped were included in the analysis in chapter 9. Some children were excluded due to age and adoption status. Colours on the gels were inverted for enhanced clarity with printing.

Gel 1

<table>
<thead>
<tr>
<th>Lane number</th>
<th>Sample number</th>
<th>Sequenced?</th>
<th>Sequencing result or score in gel</th>
</tr>
</thead>
<tbody>
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<td>Blank</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>Ladder</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 1</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 2</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 3</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 4</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Sample 5</td>
<td>Yes</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 8</td>
<td>Sample 6</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 9</td>
<td>Sample 7</td>
<td>Yes</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 10</td>
<td>Sample 8</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 11</td>
<td>Sample 9</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 12</td>
<td>Sample 10</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 13</td>
<td>Control</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Agarose gel electrophoresis results for the DAT1 3’ uVNTR: Gel 1
Gel 2

Figure 2. Agarose gel electrophoresis results for the DAT1 3' uVNTR: Gel 2

<table>
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<tr>
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<th>Sample number</th>
<th>Sequenced?</th>
<th>Sequencing result or score in gel</th>
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</thead>
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<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>Ladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 11</td>
<td>No</td>
<td>Did not amplify</td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 12</td>
<td>No</td>
<td>Unclear – rerun in gel 7 lane 4</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 13</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 14</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Sample 15</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 8</td>
<td>Sample 16</td>
<td>No</td>
<td>Unclear - Redone in gel 7 lane 5</td>
</tr>
<tr>
<td>Lane 9</td>
<td>Sample 17</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 10</td>
<td>Sample 18</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 11</td>
<td>Sample 19</td>
<td>Yes</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 12</td>
<td>Sample 20</td>
<td>No</td>
<td>Did not amplify (Rerun in gel 12 lane 2)</td>
</tr>
<tr>
<td>Lane 13</td>
<td>Control</td>
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</tbody>
</table>
Gel 3

Figure 3. Agarose gel electrophoresis results for the DAT1 3’ uVNTR: Gel 3

<table>
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<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>Ladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 21</td>
<td>No</td>
<td>Did not amplify (Rerun in gel 12 lane 3)</td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 22</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 23</td>
<td>Yes</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 24</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Sample 25</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 8</td>
<td>Sample 26</td>
<td>No</td>
<td>Unclear – rerun in gel 12 lane 4</td>
</tr>
<tr>
<td>Lane 9</td>
<td>Sample 27</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 10</td>
<td>Sample 28</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 11</td>
<td>Sample 29</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 12</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Gel 4

![Agarose gel electrophoresis results for the DAT1 3' uVNTR: Gel 4](image)

<table>
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<tr>
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<th>Sequencing result or score in gel</th>
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</thead>
<tbody>
<tr>
<td>Lane 1</td>
<td>Blank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>Ladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 31</td>
<td>No</td>
<td>Did not amplify (Rerun in gel 12 lane 5)</td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 32</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 33</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 34</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Sample 35</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 8</td>
<td>Sample 36</td>
<td>No</td>
<td>Unclear – rerun in gel 10 lane 6</td>
</tr>
<tr>
<td>Lane 9</td>
<td>Sample 37</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 10</td>
<td>Sample 38</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 11</td>
<td>Sample 39</td>
<td>No</td>
<td>Unclear – rerun in gel 12 lane 6</td>
</tr>
<tr>
<td>Lane 12</td>
<td>Sample 40</td>
<td>No</td>
<td>10 homozygote – confirmed in gel 11 lane 3</td>
</tr>
<tr>
<td>Lane 13</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Gel 5

*Figure 5. Agarose gel electrophoresis results for the DAT1 3' uVNTR: Gel 5*

<table>
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<tr>
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<th>Sample number</th>
<th>Sequenced?</th>
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<tbody>
<tr>
<td>Lane 1</td>
<td>Ladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>Sample 41</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 42</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 43</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 44</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 45</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Sample 46</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 8</td>
<td>Sample 47</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 9</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Figure 6. Agarose gel electrophoresis results for the DAT1 3’ uVNTR: Gel 6**

<table>
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<tr>
<th>Lane number</th>
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</tr>
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<tbody>
<tr>
<td>Lane 1</td>
<td>Ladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>Sample 48</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 49</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 50</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 51</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 52</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Gel 7

Figure 7. Agarose gel electrophoresis results for the DAT1 3' uVNTR: Gel 7

<table>
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<tr>
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<th>Sample number</th>
<th>Sequenced?</th>
<th>Sequencing result or score in gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lane 1</td>
<td>Ladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>Sample 53</td>
<td>Yes</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 54</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 12 (Redone from gel 2 lane 4)</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 16 (Redone from gel 2 lane 8)</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 55</td>
<td>Yes</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Sample 56</td>
<td>Yes</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 8</td>
<td>Control</td>
<td></td>
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</tr>
</tbody>
</table>
Figure 8. Agarose gel electrophoresis results for the DAT1 3' uVNTR: Gel 8

<table>
<thead>
<tr>
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<th>Sample number</th>
<th>Sequenced?</th>
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<tbody>
<tr>
<td>Lane 1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>Sample 57</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 58</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 59</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 60</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 61</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Sample 62</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 8</td>
<td>Control</td>
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</table>
Figure 9. Agarose gel electrophoresis results for the DAT1 3’ uVNTR: Gel 9

<table>
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<tr>
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<th>Sample number</th>
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<th>Sequencing result or score in gel</th>
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<tr>
<td>Lane 2</td>
<td>Sample 63</td>
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<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 64</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
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<tr>
<td>Lane 4</td>
<td>Sample 65</td>
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<td>10 homozygote</td>
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<tr>
<td>Lane 5</td>
<td>Sample 66</td>
<td>No</td>
<td>9 homozygote</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 67</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Sample 68</td>
<td>No</td>
<td>9/10 heterozygote</td>
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<tr>
<td>Lane 8</td>
<td>Control</td>
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</tbody>
</table>
### Gel 10

![Agarose gel electrophoresis results for the DAT1 3’ uVNTR: Gel 10](image)

<table>
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<th>Sequencing result or score in gel</th>
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<td>Lane 1</td>
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</tr>
<tr>
<td>Lane 2</td>
<td>Sample 69</td>
<td>No</td>
<td>10 homozygote</td>
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<tr>
<td>Lane 3</td>
<td>Sample 70</td>
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<td>Lane 4</td>
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<td>Lane 5</td>
<td>Sample 72</td>
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<td>10 homozygote</td>
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<td>Sample 36 (Redone from gel 4)</td>
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<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Sample 73</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 8</td>
<td>Control</td>
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</tr>
</tbody>
</table>
### Gel 11

*Figure 11. Agarose gel electrophoresis results for the DAT1 3’ uVNTR: Gel 11*

<table>
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<td>Lane 1</td>
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</tr>
<tr>
<td>Lane 2</td>
<td>Sample 74</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 40</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td></td>
<td>(Confirmed from Gel 4 lane 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 75</td>
<td>No</td>
<td>10 homozygote</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 76</td>
<td>No</td>
<td>Did not amplify (Too little DNA to rerun)</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 77</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 12. Agarose gel electrophoresis results for the DAT1 3’ uVNTR: Gel 12

<table>
<thead>
<tr>
<th>Lane number</th>
<th>Sample number</th>
<th>Sequenced?</th>
<th>Sequencing result or score in gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lane 1</td>
<td>Ladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>Sample 20 (Rerun from gel 2 lane 12)</td>
<td>No</td>
<td>Did not amplify</td>
</tr>
<tr>
<td>Lane 3</td>
<td>Sample 21 (Rerun from gel 3 lane 3)</td>
<td>No</td>
<td>Did not amplify</td>
</tr>
<tr>
<td>Lane 4</td>
<td>Sample 26 (Rerun from gel 3 lane 8)</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 5</td>
<td>Sample 31 (Rerun from gel 4 lane 3)</td>
<td>No</td>
<td>Did not amplify</td>
</tr>
<tr>
<td>Lane 6</td>
<td>Sample 39 (Rerun from gel 4 lane 11)</td>
<td>No</td>
<td>9/10 heterozygote</td>
</tr>
<tr>
<td>Lane 7</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix C2: Sequencing results for the polymorphism in the DAT1 gene investigated in chapter 9 (DAT1 3’ uVNTR)

The sequences obtained for the different samples that were sequenced for the DAT1 3’ uVNTR are presented below. Full repeats are highlighted with red and grey consecutively so that the number of repeats can be counted.

*Note: Unclear peaks were marked with the number N.

Sample 5: 9 repeats

<table>
<thead>
<tr>
<th>Sample 5</th>
<th>9 repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAGGCTGTCCTAT</td>
<td>GAGGCTGTCCTAT</td>
</tr>
<tr>
<td>CAGGACCTGACGAGGAG</td>
<td>CAGGACCTGACGAGGAG</td>
</tr>
<tr>
<td>GAGCATGTCAGGGCCCATG</td>
<td>GAGCATGTCAGGGCCCATG</td>
</tr>
<tr>
<td>GAGCGTGTACTACCCAGGACGCATGCAGGGCCCCCACAG</td>
<td>GAGCGTGTACTACCCAGGACGCATGCAGGGCCCCCACAG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
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</table>

Sample 7: 9 repeats

<table>
<thead>
<tr>
<th>Sample 7</th>
<th>9 repeats</th>
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</thead>
<tbody>
<tr>
<td>GAGGACCTGACGAGGAG</td>
<td>GAGGACCTGACGAGGAG</td>
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<td>CAGGACCTGACGAGGAG</td>
<td>CAGGACCTGACGAGGAG</td>
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<td>GAGCATGTCAGGGCCCATG</td>
<td>GAGCATGTCAGGGCCCATG</td>
</tr>
<tr>
<td>GAGCGTGTACTACCCAGGACGCATGCAGGGCCCCCACAG</td>
<td>GAGCGTGTACTACCCAGGACGCATGCAGGGCCCCCACAG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
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<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
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<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
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<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
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<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
</tbody>
</table>

Sample 53: 10 repeats

<table>
<thead>
<tr>
<th>Sample 53</th>
<th>10 repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAGGACCTGACGAGGAG</td>
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<td>CAGGACCTGACGAGGAG</td>
<td>CAGGACCTGACGAGGAG</td>
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<tr>
<td>GAGCATGTCAGGGCCCATG</td>
<td>GAGCATGTCAGGGCCCATG</td>
</tr>
<tr>
<td>GAGCGTGTACTACCCAGGACGCATGCAGGGCCCCCACAG</td>
<td>GAGCGTGTACTACCCAGGACGCATGCAGGGCCCCCACAG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
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<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
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</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
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<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
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<tr>
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<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
<tr>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
<td>GAGGCTGTCCTATCCCCGGACCGGACGCATG</td>
</tr>
</tbody>
</table>

*Note: For this sample, the sequencing picked up near the end of the first repeat, but taken together with the position of the band in the gel, it was clear that the sample consisted of 10 repeats of the 40 base pair repeat sequence.
Sample 23: 9 repeats
GGGAAGGCGGGAGAG AAGCGTGTCTNNNNCCCCGGANNNANNCAGGGCCCCCAGAG GAGCGTGTCTCTATGC
CCCGGAGCGCATGAGGCCCCCAGAG GAGCGTGTCTCTATCCGGAGCGCATGAGGGCCCCCAGAG GAGCGT
GTACTACACGACGATGCAGGCCCCCAGAG AAGCGTGTACTCCCCAGAGGCGATGCAGGCCCCCAGAG GAGCGT
GTACTACACGACGATGCAGGCCCCCAGAG AAGCGTGTACTCCCCAGAGGCGATGCAGGCCCCCAGAG GAGCGT
CTACGACGATGCAGGCCCCCAGAG AAGCGTGTACTCCCCAGAGGCGATGCAGGCCCCCAGAG GAGCGT
GTACTACACGACGATGCAGGCCCCCAGAG AAGCGTGTACTCCCCAGAGGCGATGCAGGCCCCCAGAG GAGCGT
GTACGACGATGCAGGCCCCCAGAG AAGCGTGTACTCCCCAGAGGCGATGCAGGCCCCCAGAG GAGCGT
Sample 55: 9 repeats
*Note: For this sample, the sequencing picked up near the middle of the first repeat, but taken together with the position of the band in the gel, it was clear that the sample consisted of 9 repeats of the 40 base pair repeat sequence.

Sample 56: 10 repeats
*Note: For this sample, the sequencing picked up near the end of the first repeat, but taken together with the position of the band in the gel, it was clear that the sample consisted of 10 repeats of the 40 base pair repeat sequence.
Appendix D: Genotyping results for chapter 10

Appendix D1: Agarose gel electrophoresis results for the polymorphism in the MAOA gene investigated in chapter 10 (MAOA-uVNTR)

*Note: Since all homozygote samples were sequenced, and all heterozygote samples were not considered, no scoring of the samples in the gels were conducted. The gels were simply run to identify homozygote samples for the sequencing reactions. Since only children between the ages of 5 to 18, and who were formally diagnosed with ADHD, were considered for chapter 10, there are more homozygote samples in the gels than are presented in chapter 10.

Gel 1

Figure 13. Agarose gel electrophoresis results for the MAOA-uVNTR: Gel 1

Gel 2

Figure 14. Agarose gel electrophoresis results for the MAOA-uVNTR: Gel 2
Figure 15. Agarose gel electrophoresis results for the MAOA-uVNTR: Gel 3

Figure 16. Agarose gel electrophoresis results for the MAOA-uVNTR: Gel 4

Figure 17. Agarose gel electrophoresis results for the MAOA-uVNTR: Gel 5
Figure 18. Agarose gel electrophoresis results for the MAOA-uVNTR: Gel 6
Appendix D2: Sequencing results for the polymorphism in the MAOA gene investigated in chapter 10 (MAOA-uVNTR)

The sequences obtained for the different samples following sequencing are presented below. Full repeats are highlighted in red and grey consecutively, to enable counting of the number of repeats present. Half repeats are highlighted in blue. Rare mutational events are highlighted in yellow. Since only children between the ages of 5 to 18, and who were formally diagnosed with ADHD, were considered for chapter 10, there are more sequences presented here than the number of participant in chapter 10.

Sample 1: 3.5 repeats

ACAGCCTGAGTGGAGAAGGGCTCGGGGAAAGCAGAAACACCACCAGCCGACCGGTGCTCCAGAACA
TGAGCACAAGGCTCAGCTCCCTCTCCCAGGCGGCACCGACCGGCACCACGCCAGTACCCCGCACGATACCCGGCA
CCGGCACCACTACCACCCGACCAGTACCCGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGCA
CAAGGCGAGGCGCCCGCAAGGGGAGCACAATCTCCAGTGACCAGCTTGGACGACACCTCCTACAGCCTGTCCGAATGGAGCGTCCGGTCA

Sample 2: 4.5 repeats

TACAGCCTGAGTGGAGAAGGGCTCGGGGAAAGCAGAAACACCACCAGCCGACCGGTGCTCCAGAACA
TGAGCACAAGGCTCAGCTCCCTCTCCCAGGCGGCACCGACCGGCACCACGCCAGTACCCCGCACGATACCCGGCA
CCGGCACCACTACCACCCGACCAGTACCCGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGCA
CAAGGCGAGGCGCCCGCAAGGGGAGCACAATCTCCAGTGACCAGCTTGGACGACACCTCCTACAGCCTGTCCGAATGGAGCGTCCGGTCA

Sample 3: 4.5 repeats

TACAGCCTGAGTGGAGAAGGGCTCGGGGAAAGCAGAAACACCACCAGCCGACCGGTGCTCCAGAACA
TGAGCACAAGGCTCAGCTCCCTCTCCCAGGCGGCACCGACCGGCACCACGCCAGTACCCCGCACGATACCCGGCA
CCGGCACCACTACCACCCGACCAGTACCCGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGCA
CAAGGCGAGGCGCCCGCAAGGGGAGCACAATCTCCAGTGACCAGCTTGGACGACACCTCCTACAGCCTGTCCGAATGGAGCGTCCGGTCA

Sample 4: 4.5 repeats

ACAGCCTGAGTGGAGAAGGGCTCGGGGAAAGCAGAAACACCACCAGCCGACCGGTGCTCCAGAACA
TGAGCACAAGGCTCAGCTCCCTCTCCCAGGCGGCACCGACCGGCACCACGCCAGTACCCCGCACGATACCCGGCA
CCGGCACCACTACCACCCGACCAGTACCCGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGCA
CAAGGCGAGGCGCCCGCAAGGGGAGCACAATCTCCAGTGACCAGCTTGGACGACACCTCCTACAGCCTGTCCGAATGGAGCGTCCGGTCA

Sample 5: 4.5 repeats

TACAGCCTGAGTGGAGAAGGGCTCGGGGAAAGCAGAAACACCACCAGCCGACCGGTGCTCCAGAACA
TGAGCACAAGGCTCAGCTCCCTCTCCCAGGCGGCACCGACCGGCACCACGCCAGTACCCCGCACGATACCCGGCA
CCGGCACCACTACCACCCGACCAGTACCCGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGACCAGTACCCGGCA
CAAGGCGAGGCGCCCGCAAGGGGAGCACAATCTCCAGTGACCAGCTTGGACGACACCTCCTACAGCCTGTCCGAATGGAGCGTCCGGTCA
Appendices / 297

Sample 6: 3.5 repeats
TACAGCCTGACCGTGGAGAAGGGCTGCGGGAAGCAGAACACCGCCCCCAGCGCCCAGCGTGCTCCAGAAAC
ATGAGCACAAAACGCCTAGCCTCTCTCTCCTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
GCAGGACACCGGACTCGCTGAGACACCGGGATCGGCCAGGTCGTCCAGTTCA

Sample 7: 4.5 repeats
TACAGCCTGACCGTGGAGAAGGGCTGCGGGAAGCAGAACACCGCCCCCAGCGCCCAGCGTGCTCCAGAAAC
ATGAGCACAAAACGCCTAGCCTCTCTCTCCTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
GCAGGACACCGGACTCGCTGAGACACCGGGATCGGCCAGGTCGTCCAGTTCA

Sample 8: 3.5 repeats
TACAGCCTGACCGTGGAGAAGGGCTGCGGGAAGCAGAACACCGCCCCCAGCGCCCAGCGTGCTCCAGAAAC
ATGAGCACAAAACGCCTAGCCTCTCTCTCCTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
GCAGGACACCGGACTCGCTGAGACACCGGGATCGGCCAGGTCGTCCAGTTCA

Sample 9: 4.5 repeats
TACAGCCTGACCGTGGAGAAGGGCTGCGGGAAGCAGAACACCGCCCCCAGCGCCCAGCGTGCTCCAGAAAC
ATGAGCACAAAACGCCTAGCCTCTCTCTCCTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
GCAGGACACCGGACTCGCTGAGACACCGGGATCGGCCAGGTCGTCCAGTTCA

Sample 10: 4.5 repeats
ACAGCCTGACCGTGGAGAAGGGCTGCGGGAAGCAGAACACCGCCCCCAGCGCCCAGCGTGCTCCAGAAAC
TGAGCACAAAACGCCTAGCCTCTCTCTCCTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
CCGGCACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
GCAGGACACCGGACTCGCTGAGACACCGGGATCGGCCAGGTCGTCCAGTTCA

Sample 11: 4.5 repeats
TGACCGTGGAAGGCGCTGGGAAGGACGAGAAACACCGCCCCCAGCGCCCAGCGTGCTCCAGAAAC
CAAACGCCTAGCCTCTCTCCTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCA
CCGGGACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
CCGGGACCAGTACCGGCACCAGTACCGGCA
CCGGGCACCGGCACCAGTACCGGCA
GCAGGACACCGGACTCGCTGAGACACCGGGATCGGCCAGGTCGTCCAGTTCA
Sample 12: 3.5 repeats

GTGCTCCAGAACATGAGCACAACACGTACCGCCTTCCTCTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC

Sample 13: 4.5 repeats

TACAGCCTGAGCGAGAAGGGGTCGGGGAAGCAGAACACCGCCCTTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC

Sample 14: 4.5 repeats

GAGAAGGGGTCGGGGAAGCAGAACACCGCCCTTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC

Sample 15: 3.5 repeats

TGACCGTGGAGAAGGGGTCGGGGAAGCAGAACACCGCCCTTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC

Sample 16: 3.5 repeats

CAAAACGCCCTAGCTGGGGAAGCAGAACACCGCCCTTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC

Sample 17: 3.5 repeats

TACAGCCTGAGCGAGAAGGGGTCGGGGAAGCAGAACACCGCCCTTCCCCGGCGGC
ACCGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC
ACCAGT ACCGGGCACCGGCACCAGTACCCGC

Appendices

Sample 18: 3.5 repeats
TACAGCTGACCGTGGAGAAGGCTGCGGAAGCACACCGCCCCCAGCCAGCGCTGCTCGAAC
ATGAGCAAAAGCCTCAGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
**Sample 24: 4.5 repeats**

CCGTGGAGAGGGCGGTGGAGGGAGCAAAACACCGCCCCACCCAGGGCCACGGATGCTCCAGA

**Sample 25: 4.5 repeats**

TGACCGTGGAGAGGGCTGGAGAGCAACCCCGCCCCACCCAGGGCCACGGATGCTCCAGAA

**Sample 26: 4.5 repeats**

ACAGCCTGAGCCACCAAGGGCTGGAGAGCAACCCCGCCCCACCCAGGGCCACGGATGCTCCAGAA

**Sample 27: 3.5 repeats**

TACAGCCTGAGCCACCAAGGGCTGGAGAGCAACCCCGCCCCACCCAGGGCCACGGATGCTCCAGAA

**Sample 28: Rare mutation**

– Deletion of ACCCGCACGT in fourth repeat, followed by a half repeat

**Sample 29: Rare mutation**

– Deletion of ACCCGCACGT in fourth repeat, followed by a half repeat
Sample 29: Rerun to assure presence of mutation
TACAGCCTGACCGTGGAGAGGCTCGGGAAGCAGAACACGCACCAGCAGCGTGCTCCAGAAA
ATGAGCATAAACAGCTAGCCTAGCTCCTTCCTCCCCGGGACCAGGAGACACCGGCACCGACCAGGCACCGGAGGCGCTGC
TACCGGACCAGGCAGCGACTCCAGCTTGGACGACACCGCTTTAGCCAGAATGGAGCGTCCGTTCA

Sample 30: Rare mutation – Deletion of ACCGGCACCAGT in fourth repeat, followed by a half repeat
TTACAGCCTGACCGTGGAGAGGCTCGGGAAGCAGAACACGCACCAGCAGCGTGCTCCAGAAA
ATGAGCATAAACAGCTAGCCTAGCCTAGCTCCTTCCTCCCCGGGACCAGGAGACACCGGCACCGACCAGGCACCGGAGGCGCTGC
TACCGGACCAGGCAGCGACTCCAGCTTGGACGACACCGCTTTAGCCAGAATGGAGCGTCCGTTCA

Sample 31: 4 repeats
TACAGCCTGACCGTGGAGAGGCTCGGGAAGCAGAACACGCACCAGCAGCGTGCTCCAGAAA
ATGAGCATAAACAGCTAGCCTAGCCTAGCTCCTTCCTCCCCGGGACCAGGAGACACCGGCACCGACCAGGCACCGGAGGCGCTGC
TACCGGACCAGGCAGCGACTCCAGCTTGGACGACACCGCTTTAGCCAGAATGGAGCGTCCGTTCA

Sample 32: 3.5 repeats
TACAGCCTGACCGTGGAGAGGCTCGGGAAGCAGAACACGCACCAGCAGCGTGCTCCAGAAA
ATGAGCATAAACAGCTAGCCTAGCCTAGCTCCTTCCTCCCCGGGACCAGGAGACACCGGCACCGACCAGGCACCGGAGGCGCTGC
TACCGGACCAGGCAGCGACTCCAGCTTGGACGACACCGCTTTAGCCAGAATGGAGCGTCCGTTCA

Sample 33: 5.5 repeats
TACAGCCTGACCGTGGAGAGGCTCGGGAAGCAGAACACGCACCAGCAGCGTGCTCCAGAAA
ATGAGCATAAACAGCTAGCCTAGCCTAGCTCCTTCCTCCCCGGGACCAGGAGACACCGGCACCGACCAGGCACCGGAGGCGCTGC
TACCGGACCAGGCAGCGACTCCAGCTTGGACGACACCGCTTTAGCCAGAATGGAGCGTCCGTTCA
Sample 34: 3.5 repeats
TACAGCCTGACCGTGGAGAAGGGCTGCGGGAAGCAGAACACCGCCCCCAGCGCCCAGCGTGCTCCAGAAAC
ATGAGCACAAACGCCTCAGCCTCCTTCCCCGGCCGGCAACCACCGACCCACGACCGCTGCTCCAGAAAC
CCCGGCA
Sample 35: 4.5 repeats
TACAGCCTGACCGTGGAGAAGGGCTGCGGGAAGCAGAACACCGCCCCCAGCGCCCAGCGTGCTCCAGAAAC
ATGAGCACAAACGCCTCAGCCTCCTTCCCCGGCCGGCAACCACCGACCCACGACCGCTGCTCCAGAAAC
CCCGGCA
Sample 36: 4.5 repeats
TACAGCCTGACCGTGGAGAAGGGCTGCGGGAAGCAGAACACCGCCCCCAGCGCCCAGCGTGCTCCAGAAAC
ATGAGCACAAACGCCTCAGCCTCCTTCCCCGGCCGGCAACCACCGACCCACGACCGCTGCTCCAGAAAC
CCCGGCA
Appendix E: Extracts of statistical analyses

Appendix E1: Extracts of the statistical analysis results for the chapter titled “Familial aggregation of Attention-Deficit/Hyperactivity Disorder (ADHD) subtypes in a South African family study (chapter 4)

*Note: For space and printing considerations, only extracts of the statistical analysis are presented here.

Multilevel models for hyperactivity-impulsivity: Comparison of model where family structure is not taken into account with model where family structure is taken into account

Family structure not taken into account:

Model Dimension

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>Number of Levels</th>
<th>Number of Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gender</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Home_Language_num</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Residual:

1

Total:

8

7

Information Criteria

-2 Log Likelihood

<table>
<thead>
<tr>
<th>Information Criterion</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akaike's Information Criterion (AIC)</td>
<td>575.124</td>
</tr>
<tr>
<td>Hurvich and Tsai's Criterion (AICC)</td>
<td>589.124</td>
</tr>
<tr>
<td>Bozdogan's Criterion (CAIC)</td>
<td>589.687</td>
</tr>
<tr>
<td>Schwarz's Bayesian Criterion (BIC)</td>
<td>619.453</td>
</tr>
<tr>
<td>Schwarz's Bayesian Criterion (BIC)</td>
<td>612.453</td>
</tr>
</tbody>
</table>

a. Dependent Variable: Zscore(ADHD_Hyperact_total_probands).

The information criteria are displayed in smaller-is-better form.

a. Dependent Variable: Zscore(ADHD_Hyperact_total_probands).
Family structure taken into account

<table>
<thead>
<tr>
<th>Model Dimension(a)</th>
<th>Number of Levels</th>
<th>Covariance Structure</th>
<th>Number of Parameters</th>
<th>Subject Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed Effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>2</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Home_Language_num</td>
<td>4</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Random Effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
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<td>1</td>
<td>Family_num</td>
</tr>
<tr>
<td>Intercept(b)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td></td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

\(a\). Dependent Variable: \(Zscore(ADHD\_Hyperact\_total\_probands)\).

<table>
<thead>
<tr>
<th>Information Criteria(a)</th>
<th>(\text{(-2 Log Likelihood)})</th>
<th>(\text{(573.147)})</th>
<th>(\text{(589.147)})</th>
<th>(\text{(589.874)})</th>
<th>(\text{(623.809)})</th>
<th>(\text{(615.809)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akaike’s Information</td>
<td>(\text{\text{Criterion (AIC)}})</td>
<td>(\text{(573.147)})</td>
<td>(\text{(589.147)})</td>
<td>(\text{(589.874)})</td>
<td>(\text{(623.809)})</td>
<td>(\text{(615.809)})</td>
</tr>
<tr>
<td>Hurvich and Tsai’s</td>
<td>Criterion (AICC)</td>
<td>(\text{(573.147)})</td>
<td>(\text{(589.147)})</td>
<td>(\text{(589.874)})</td>
<td>(\text{(623.809)})</td>
<td>(\text{(615.809)})</td>
</tr>
<tr>
<td>Bozdogan's Criterion</td>
<td>(CAIC)</td>
<td>(\text{(573.147)})</td>
<td>(\text{(589.147)})</td>
<td>(\text{(589.874)})</td>
<td>(\text{(623.809)})</td>
<td>(\text{(615.809)})</td>
</tr>
<tr>
<td>Schwarz’s Bayesian</td>
<td>Criterion (BIC)</td>
<td>(\text{(573.147)})</td>
<td>(\text{(589.147)})</td>
<td>(\text{(589.874)})</td>
<td>(\text{(623.809)})</td>
<td>(\text{(615.809)})</td>
</tr>
</tbody>
</table>

The information criteria are displayed in smaller-is-better form.

\(a\). Dependent Variable: \(Zscore(ADHD\_Hyperact\_total\_probands)\).

\(\text{\(\text{Chi-square change} = 575.124 - 573.147\)}\)

\(= \ 1.977\)

\(\text{\(\text{Degrees of freedom} = 1\)}\)

\(\text{\(\text{Result} = \text{No significant difference}\)}\)
Multilevel models for inattention: Comparison of model where family structure is not taken into account with model where family structure is taken into account

Family structure not taken into account:

**Model Dimension**

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>Number of Levels</th>
<th>Number of Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gender</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Home-Language_num</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8</strong></td>
<td><strong>7</strong></td>
</tr>
</tbody>
</table>

*a. Dependent Variable: Zscore(ADHD_proband_Inattention).*

**Information Criteria**

<table>
<thead>
<tr>
<th>-2 Log Likelihood</th>
<th>576.180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akaike's Information Criterion (AIC)</td>
<td>590.180</td>
</tr>
<tr>
<td>Hurvich and Tsai's Criterion (AICC)</td>
<td>590.746</td>
</tr>
<tr>
<td>Bozdogan's Criterion (CAIC)</td>
<td>620.475</td>
</tr>
<tr>
<td>Schwarz's Bayesian Criterion (BIC)</td>
<td>613.475</td>
</tr>
</tbody>
</table>

The information criteria are displayed in smaller-is-better form.

*a. Dependent Variable: Zscore(ADHD_proband_Inattention).*
### Model Dimension

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>Number of Levels</th>
<th>Covariance Structure</th>
<th>Number of Parameters</th>
<th>Subject Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>2</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Home_Language_num</td>
<td>4</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Age</td>
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<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Age Intercept</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: Zscore(ADHD_proband_Inattention).

### Information Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2 Log Likelihood</td>
<td>570.333</td>
</tr>
<tr>
<td>Akaike's Information Criterion (AIC)</td>
<td>586.333</td>
</tr>
<tr>
<td>Hurvich and Tsai's Criterion (AICC)</td>
<td>587.064</td>
</tr>
<tr>
<td>Bozdogan's Criterion (CAIC)</td>
<td>620.956</td>
</tr>
<tr>
<td>Schwarz's Bayesian Criterion (BIC)</td>
<td>612.956</td>
</tr>
</tbody>
</table>

The information criteria are displayed in smaller-is-better form.

a. Dependent Variable: Zscore(ADHD_proband_Inattention).

Chi-square change = 576.180 – 570.333 = 5.847

Degrees of freedom = 1

Result = Significant at the 5% level
Appendix E2: Extracts of the statistical analysis results for the chapter titled “Are ADHD combined type and predominantly inattentive type distinct disorders or varying presentations of the same disorder? Perspectives from a family study in a South African sample” (chapter 5)

*Note: For space and printing considerations, only extracts of the statistical analysis are presented here.

### Chi-square goodness of fit test (Inattention subtype)

<table>
<thead>
<tr>
<th>Concordance_Discordance_InattentionOnly</th>
<th>Observed N</th>
<th>Expected N</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant for Inattention</td>
<td>1</td>
<td>7.5</td>
<td>-6.5</td>
</tr>
<tr>
<td>Discordant (Hyperactivity/impulsivity and inattention plus predominantly inattention)</td>
<td>14</td>
<td>7.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Test Statistics**

<table>
<thead>
<tr>
<th>Concordance_Discordance_InattentionOnly</th>
<th>Concordance_Discordance_InattentionOnly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square</td>
<td>11.267&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>df</td>
<td>1</td>
</tr>
<tr>
<td>Asymp. Sig.</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### Chi-square goodness of fit test (Combined subtype)

<table>
<thead>
<tr>
<th>Concordance_Discordance_HandInattention</th>
<th>Observed N</th>
<th>Expected N</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant for Hyperactivity/impulsivity and Inattention</td>
<td>17</td>
<td>15.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Discordant (Hyperactivity/impulsivity and inattention plus predominantly inattention)</td>
<td>14</td>
<td>15.5</td>
<td>-1.5</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Test Statistics**

<table>
<thead>
<tr>
<th>Concordance_Discordance_HandInattention</th>
<th>Concordance_Discordance_HandInattention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square</td>
<td>.290&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>df</td>
<td>1</td>
</tr>
<tr>
<td>Asymp. Sig.</td>
<td>0.590</td>
</tr>
</tbody>
</table>

<sup>a</sup> 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 15.5.
Appendix E3: Extracts of the statistical analysis results for the chapter titled “Examining the aetiology of Attention-Deficit/Hyperactivity Disorder and Oppositional Defiant Disorder in a genetically informative sample from South Africa (chapter 6)

*Note: For space and printing considerations, only extracts of the statistical analysis are presented here.

Multinomial logistic regression ran in STATA

```
   . mlogit ADHDplusODD_Sib1 ADHDplusODD_proband, baseoutcome(1) vce(cluster Family) rrr
```

<table>
<thead>
<tr>
<th>Multinomial logistic regression</th>
<th>Number of obs</th>
<th>=</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wald chi2(3)</td>
<td>=</td>
<td>5.59</td>
</tr>
<tr>
<td></td>
<td>Prob &gt; chi2</td>
<td>=</td>
<td>0.1334</td>
</tr>
<tr>
<td>Log pseudolikelihood = -91.164411</td>
<td>Pseudo R2</td>
<td>=</td>
<td>0.0379</td>
</tr>
</tbody>
</table>

(Std. Err. adjusted for 74 clusters in Family)

| ADHDplusODD_Sib1 | RRR | Std. Err. | z   | P>|z| | [95% Conf. Interval] |
|------------------|-----|-----------|-----|------|----------------------|
| No_ADHD_or_ODD   |     |           |     |      |                      |
| ADHDplusODD_proband_cons | 2.769231 | 1.662091 | 1.70 | 0.090 | 0.850205 - 8.979455  |
| ADHDplusODD_proband_cons | 2.16667 | 0.789761 | 2.18 | 0.029 | 1.080663 - 4.34404  |
| ADHD_only        |     |           |     |      |                      |
| ODD_only         |     |           |     |      |                      |
| ADHDplusODD_proband_cons | 14.39999 | 17.78955 | 2.16 | 0.031 | 1.278792 - 162.1529  |
| ADHDplusODD_proband_cons | 0.0833334 | 0.873282 | -2.37 | 0.018 | 0.019686 - 0.6499674  |
| ADHD_plus_moderate_to_severe_ODD |     |           |     |      |                      |
| ADHDplusODD_proband_cons | 2.4 | 1.824329 | 1.15 | 0.249 | 0.5409803 - 10.64734  |
| ADHDplusODD_proband_cons | 0.416667 | 0.2097554 | -1.74 | 0.082 | 0.1553398 - 1.117621  |
Appendix E4: Extracts of the statistical analysis results for the chapter titled “Explaining sex differences in the prevalence of ADHD – testing two models in a South African sample of nuclear families (chapter 7)

*Note: For space and printing considerations, only extracts of the statistical analysis are presented here.

Analysis of covariance

<table>
<thead>
<tr>
<th>Between-Subjects Factors</th>
<th>Value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is there a female in family diagnosed with ADHD?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>.00</td>
<td>94</td>
</tr>
<tr>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>108</td>
</tr>
</tbody>
</table>

Descriptive Statistics

Dependent Variable: Total ADHD

<table>
<thead>
<tr>
<th>Is there a female in family diagnosed with ADHD?</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>.2521012</td>
<td>.99881734</td>
<td>94</td>
</tr>
<tr>
<td>No</td>
<td>-.2178011</td>
<td>.95257554</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>.0008663</td>
<td>.99992344</td>
<td>202</td>
</tr>
</tbody>
</table>

Tests of Between-Subjects Effects

Dependent Variable: Total ADHD

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>11.100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td>5.550</td>
<td>5.817</td>
<td>.004</td>
<td>.055</td>
</tr>
<tr>
<td>Intercept</td>
<td>.008</td>
<td>1</td>
<td>.008</td>
<td>.008</td>
<td>.929</td>
<td>.000</td>
</tr>
<tr>
<td>Age_All</td>
<td>.003</td>
<td>1</td>
<td>.003</td>
<td>.003</td>
<td>.956</td>
<td>.000</td>
</tr>
<tr>
<td>Female_in_family</td>
<td>11.090</td>
<td>1</td>
<td>11.090</td>
<td>11.623</td>
<td>.001</td>
<td>.055</td>
</tr>
<tr>
<td>Error</td>
<td>189.869</td>
<td>199</td>
<td>.954</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Corrected Total</td>
<td>200.969</td>
<td>202</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> R Squared = .055 (Adjusted R Squared = .046)
Appendix E5: Extracts of the statistical analysis results for the chapter titled “Influence of pregnancy and delivery complications on ADHD symptom severity in children” (chapter 8)

*Note: For space and printing considerations, only extracts of the statistical analysis are presented here.

**Generalised estimating equations (Main effects model: Inattention)**

**Model Information**

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>ADHD_proband_Inattention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability Distribution</td>
<td>Normal</td>
</tr>
<tr>
<td>Link Function</td>
<td>Identity</td>
</tr>
<tr>
<td>Subject Effect</td>
<td>1</td>
</tr>
<tr>
<td>Family_ID</td>
<td></td>
</tr>
<tr>
<td>Within-Subject Effect</td>
<td>1</td>
</tr>
<tr>
<td>Family_member_ID</td>
<td></td>
</tr>
<tr>
<td>Working Correlation Matrix Structure</td>
<td>Independent</td>
</tr>
</tbody>
</table>

**Tests of Model Effects**

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Wald Chi-Square</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>412.814</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Birth_Complications_Shared_Nonshared</td>
<td>11.067</td>
<td>1</td>
<td>.001</td>
</tr>
<tr>
<td>Pregnancy_Complication_Shared_Nonshared</td>
<td>.523</td>
<td>2</td>
<td>.770</td>
</tr>
</tbody>
</table>

Dependent Variable: ADHD_proband_Inattention
Model: (Intercept), Birth_Complications_Shared_Nonshared, Pregnancy_Complication_Shared_Nonshared
Generalised estimating equations (Main effects model: Hyperactivity-impulsivity)

**Model Information**

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>ADHD_Hyperact_total_probands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability Distribution</td>
<td>Normal</td>
</tr>
<tr>
<td>Link Function</td>
<td>Identity</td>
</tr>
<tr>
<td>Subject Effect</td>
<td>Family_ID</td>
</tr>
<tr>
<td>Within-Subject Effect</td>
<td>Family_member_ID</td>
</tr>
<tr>
<td>Working Correlation Matrix Structure</td>
<td>Independent</td>
</tr>
</tbody>
</table>

**Tests of Model Effects**

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Wald Chi-Square</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>276.691</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Birth_Comlications_Shared_Nonshared</td>
<td>5.936</td>
<td>1</td>
<td>0.015</td>
</tr>
<tr>
<td>Pregnancy_Complication_Shared_Nonshared</td>
<td>.377</td>
<td>2</td>
<td>0.828</td>
</tr>
</tbody>
</table>

Dependent Variable: ADHD_Hyperact_total_probands
Model: (Intercept), Birth_Comlications_Shared_Nonshared, Pregnancy_Complication_Shared_Nonshared
Appendix E6: Extracts of the statistical analysis results for the chapter titled “The influence of the interaction between a polymorphism in the dopamine transporter gene and pregnancy and/or delivery complications on the severity of ADHD symptoms in a South African sample” (chapter 9)

*Note: For space and printing considerations, only extracts of the statistical analysis are presented here.

**Two-way ANOVA (Inattention)**

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>300.583( ^a )</td>
<td>3</td>
<td>100.194</td>
<td>2.133</td>
<td>0.109</td>
<td>0.120</td>
</tr>
<tr>
<td>Intercept</td>
<td>15999.226</td>
<td>1</td>
<td>15999.226</td>
<td>340.538</td>
<td>0.000</td>
<td>0.879</td>
</tr>
<tr>
<td>DAT_10Repeat_Homozygous</td>
<td>76.934</td>
<td>1</td>
<td>76.934</td>
<td>1.638</td>
<td>0.207</td>
<td>0.034</td>
</tr>
<tr>
<td>Combo_Pregnancy_Birth_BornEarly</td>
<td>77.821</td>
<td>1</td>
<td>77.821</td>
<td>1.656</td>
<td>0.204</td>
<td>0.034</td>
</tr>
<tr>
<td>DAT_10Repeat_Homozygous *</td>
<td>281.165</td>
<td>1</td>
<td>281.165</td>
<td>5.984</td>
<td>0.018</td>
<td>0.113</td>
</tr>
<tr>
<td>Combo_Pregnancy_Birth_BornEarly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>2208.163</td>
<td>47</td>
<td>46.982</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21225.000</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
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<td>Corrected Total</td>
<td>2508.745</td>
<td>50</td>
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</tr>
</tbody>
</table>

\( ^a \) R Squared = .120 (Adjusted R Squared = .064)

**Univariate Tests**

<table>
<thead>
<tr>
<th>DAT_10Repeat_Homozygous</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 repeat homozygote or 9/10</td>
<td>55.936</td>
<td>1</td>
<td>55.936</td>
<td>1.191</td>
<td>0.281</td>
<td>0.025</td>
</tr>
<tr>
<td>heterozygote</td>
<td>2208.163</td>
<td>47</td>
<td>46.982</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 repeat homozygote</td>
<td>228.073</td>
<td>1</td>
<td>228.073</td>
<td>4.854</td>
<td>0.033</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>2208.163</td>
<td>47</td>
<td>46.982</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each F tests the simple effects of Combo_Pregnancy_Birth_BornEarly within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.
### Two-way ANOVA (Hyperactivity-impulsivity)

#### Tests of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>424.933*</td>
<td>3</td>
<td>141.644</td>
<td>2.120</td>
<td>0.110</td>
<td>0.119</td>
</tr>
<tr>
<td>Intercept</td>
<td>10639.020</td>
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<td>10639.020</td>
<td>159.234</td>
<td>0.000</td>
<td>0.772</td>
</tr>
<tr>
<td>DAT_10Repeat_Homozygous</td>
<td>0.220</td>
<td>1</td>
<td>0.220</td>
<td>0.003</td>
<td>0.954</td>
<td>0.000</td>
</tr>
<tr>
<td>Combo_Pregnancy_Birth_BornEarly</td>
<td>273.243</td>
<td>1</td>
<td>273.243</td>
<td>4.090</td>
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<td>0.080</td>
</tr>
<tr>
<td>DAT_10Repeat_Homozygous *</td>
<td>283.175</td>
<td>1</td>
<td>283.175</td>
<td>4.238</td>
<td>0.045</td>
<td>0.083</td>
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<tr>
<td>Combo_Pregnancy_Birth_BornEarly</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>3140.244</td>
<td>47</td>
<td>66.814</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16240.000</td>
<td>51</td>
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<td></td>
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<tr>
<td>Corrected Total</td>
<td>3565.176</td>
<td>50</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

a. R Squared = .119 (Adjusted R Squared = .063)
### Univariate Tests

<table>
<thead>
<tr>
<th>DAT_10Repeat_Homozygous</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 repeat homozygote or 9/10 Contrast</td>
<td>0.079</td>
<td>1</td>
<td>0.079</td>
<td>0.001</td>
<td>0.973</td>
<td>0.000</td>
</tr>
<tr>
<td>9 repeat homozygote Error</td>
<td>3140.244</td>
<td>47</td>
<td>66.814</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 repeat homozygote Contrast</td>
<td>387.564</td>
<td>1</td>
<td>387.564</td>
<td>5.801</td>
<td>0.020</td>
<td>0.110</td>
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<tr>
<td>10 repeat homozygote Error</td>
<td>3140.244</td>
<td>47</td>
<td>66.814</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each F tests the simple effects of Combo_Pregnancy_Birth_BornEarly within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

---

**Estimated Marginal Means of ADHD_Hyperact_total_probands**

![Graph showing estimated marginal means for ADHD_Hyperact_total_probands across different conditions.](image-url)

*Combo_Pregnancy_Birth_BornEarly*
Appendix F: Letter from the Health Research Ethics Committee of the University of the Free State showing that the study was given ethical approval

Research Division
Internal Post Box G40
E-mail address: EthicsFHS@ufs.ac.za
Ms M Marais
(051) 4027795
Fax (051) 6444359

Ms N LAUBSCHER
DEPT OF GENETICS
UFS
BLOEMFONTEIN

Dear Ms N Laubscher

ECUFS NR 67/2015
DEPARTMENT OF GENETICS
PROJECT TITLE: PUTATIVE GENETIC AND ENVIRONMENTAL FACTORS INFLUENCING ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD) IN A SOUTH AFRICAN SAMPLE

1. You are hereby kindly informed that, at the meeting held on 19 May 2015, the Ethics Committee approved the above project.

2. Committee guidance documents: Declaration of Helsinki, ICH, GCP and MARC Guidelines on Bio Medical Research, Clinical Trial Guidelines 2000 Department of Health RSA; Ethics in Health Research: Principles Structure and Processes Department of Health RSA 2004; Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa, Second Edition (2006); the Constitution of the Ethics Committee of the Faculty of Health Sciences and the Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines.

3. Any amendment, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

4. A progress report should be submitted within one year of approval of long term studies and a final report at completion of both short term and long term studies.

5. Kindly use the ECUFS NR as reference in correspondence to the Ethics Committee Secretariat.

Yours faithfully

DR SM LE GRANGE
CHAIR: ETHICS COMMITTEE

cc: Prof JJ Spies