Autistic traits, withdrawn behaviour and cognition

Rosa Hoekstra

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A longitudinal twin study from early childhood to young adulthood

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Hierbij nodig ik u uit voor het bijwonen van de verdediging van mijn proefschrift, getiteld:

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Rosa Hoekstra
1 Derry Mews 39a Bavaria Road
N19 4EU London
Groot Brittannië

ra.hoekstra@psy.vu.nl

Autistic Traits, Withdrawn Behaviour and Cognition: A Longitudinal Twin Study from Early Childhood to Young Adulthood

Rosa Hoekstra

Reading committee
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Prof. dr. H.A. Delemarre-van de Waal
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CHAPTER 1

General Introduction

Introduction

Why does one adolescent show great difficulties in social interaction, while some of his classmates prefer to do nothing other than meeting up with friends? What factors underlie individual differences in withdrawn behaviour? Research questions like these can be addressed using genetically informative data, such as data from twins and their siblings (Boomsma et al., 2002). By comparing the phenotypic resemblance of monozygotic (MZ) twins with the resemblance of dizygotic (DZ) twins and non-twin siblings, the genetic and environmental influences on individual differences can be disentangled, because twins and siblings share their home environment, but MZ twins differ in their genetic relatedness as compared to DZ twins and siblings. The environmental influences can be further decomposed into influences from the environment that are shared between the family members (such as the neighbourhood they grow up in, nutrition, and socioeconomic status), and influences that are unique to each family member (such as an accident or illness, and friends or school experiences unshared with the co-twin or sibling).

Throughout development, genetic and environmental influences are subject to change. Genes can be switched on or off in the course of development due to e.g. hormonal signals (Alberts et al., 1994). Moreover, the environment a child is exposed to changes substantially: During adolescence, youth from western societies spend progressively more time at school and with peers, and less time in the home environment (Larson & Verma, 1999). Hence, the relative importance of genetic, shared environmental and nonshared environmental effects to the variance in traits can change over time, urging the study of individual differences at multiple time points in life. This thesis aims to study developmental processes from early childhood to young adulthood and focuses on two domains: the aetiology of individual differences in autistic traits and withdrawn behaviour, and the development of cognitive abilities. Within this thesis, findings from both cross-sectional and longitudinal studies are discussed. Cross-sectional twin and twin family studies can give insight in the relative importance of genetic and environmental effects at certain time points in life. Besides providing insight in the development over time, longitudinal studies can also unravel the aetiology of stability in traits. Using longitudinal data, questions such as "Why does the one child continue to show problem behaviour, while another "grows over" it?" or "What factors cause stability in cognitive abilities over time?" can be addressed.

Autistic traits in the general population

Autism is characterised by abnormalities in social and communication development and the presence of restricted repetitive behaviour and activities (American Psychiatric Association, 2000). Twin and family studies have shown that genetic influences play a major role in the risk for autism, and it is now thought that autism is one of the most heritable disorders in psychopathology, with a heritability above 90% (Rutter, 2000). Twin and family studies have also demonstrated that having a relative with autism not only increases the risk for a clinical diagnosis for autism, but also induces the expression of milder, but qualitatively similar autistic traits, such as social and communication difficulties, language deficits, circumscribed interests and difficulty with change (Bailey et al., 1998; Bolton et al., 1994; Landa et al., 1992; Piven et al., 1997a; Piven et al., 1997b). These findings lead to the hypothesis that the genetic variants influencing the risk for clinical autism may also affect the expression of less severe sub clinical autistic traits (Constantino et al., 2006; Piven et al., 1997a; Spiker et al., 2002). Rather than a distinct disorder, it is now thought that autism represents the upper extreme of a constellation of autistic traits, that may be continuously distributed in the general population (Constantino & Todd, 2003; Piven et al., 1997a; Spiker et al., 2002).

Using a dimensional approach, studies in 7- to 15-year-old twins showed that autistic traits are moderately to highly heritable in the general population in middle childhood and early adolescence (Constantino & Todd, 2000; Constantino & Todd, 2003; Ronald et al., 2005; Ronald et al., 2006). So far, no studies in more advanced stages of development have been reported, and none have included siblings of the twins. Moreover, all studies up to date relied on parent- or teacher-reported endorsement of autistic traits, using questionnaires in which the parent or teacher was asked to rate the child's behaviour. No twin studies using self-report measures of autistic traits have been performed.

In 2001, Baron-Cohen and colleagues developed the Autism-Spectrum Quotient (AQ), a self-report instrument to quantify autistic traits in individuals with normal intelligence (Baron-Cohen et al., 2001). Studies in Britain and Japan showed elevated scores on the AQ in individuals with an autism spectrum diagnosis (Baron-Cohen et al., 2001; Wakabayashi et al., 2006) and reported good test-retest reliability and moderate internal consistency of the AQ (Baron-Cohen et al., 2001; Kurita et al., 2005). However, the test characteristics for the Dutch translation of the AQ were unknown. Furthermore, studies into the factor structure of the AQ were lacking, and it was unknown whether elevated AQ scores are specific to individuals with autism, or could also apply to individuals with other psychiatric disorders. This thesis starts off with an examination of the test characteristics of the Dutch translation of the AQ, its factor structure and diagnostic validity. After having established the validity and

reliability of the AQ, the heritability of autistic traits as measured using self-report AQ scores is assessed in a general population sample of 18-year-old twins and their siblings. Furthermore, the degree of partner resemblance in endorsement of autistic traits in the general population is explored. If present in the general population, assortative mating could affect the frequency of the genotypes associated to autistic traits and could consequently bias the estimate of genetic influences.

Autistic traits and withdrawn behavioural problems

Individuals with a clinical diagnosis for autism often show additional behavioural problems other than the core symptoms for autism. Clinical studies indicate an increased prevalence of affective disorder, phobia, obsessive compulsive disorder, and attention-deficit hyperactivity disorder in individuals with autism (Howlin, 2000; Lainhart & Folstein, 1994; Leyfer et al., 2006; Matson & Nebel-Schwalm, 2006). Relatives of children with autism also seem to be at increased risk for major depression, anxiety, social phobia and obsessive compulsive disorder (Bolton et al., 1998; Micali et al., 2004; Piven & Palmer, 1999; Smalley et al., 1995). However, whether these elevated risks can be partly explained by the burden of caring for an autistic child, or are due to genetic risk factors shared with the risk for autism is unclear. In this thesis, the covariation between autistic traits and a broad range of behavioural problems (as assessed using Youth Self Report (YSR) ratings (Achenbach & Rescorla, 2001; Verhulst et al., 1997) is explored. Furthermore, it is examined whether the observed covariation is of genetic or environmental origin, using multivariate genetic analyses.

After having examined the aetiology of self-reported problem behaviours in 18-year-old twins and their siblings using scores on the YSR, we aimed to study the development of behavioural problems in an earlier phase of life. We wished to focus on the development of withdrawn behavioural problems, as previous studies showed that withdrawn behaviour and behavioural inhibition are fairly common in childhood (Kagan et al., 1988) and are predictive of psychiatric disorders later in life (Caspi et al., 1996; Goodwin et al., 2004). The Netherlands Twin Register (NTR) has a long history in collecting questionnaire data concerning childhood behavioural problems (Bartels et al., 2007), using parental ratings of the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001; Verhulst et al., 1996). The CBCL and YSR are developed in parallel. Using these questionnaires in concert can provide insight in the aetiology of problem behaviours in different phases of development. This thesis reports on the development of withdrawn behavioural problems in childhood, by analysing CBCL data collected when the twins were 3, 7, 10, and 12 years old. Previous studies have shown that different raters provide different information about a child's behaviour (Bartels et al., 2003; Bartels et al., 2004). As the NTR collects both maternal and

paternal ratings of the twins' behaviour, we could distinguish between the variance shared between the parents (representing the perception of the child's behaviour that both parent agree on), and the variance specific to each rater.

The development of cognitive abilities

One of the best studied domains in behaviour genetics is general cognitive ability, or intelligence. Both twin and adoption studies have examined genetic and environmental influences on the variance of general intelligence at multiple time points in development. While shared environmental influences explain about half of the variance in general cognitive abilities in young children, these influences gradually decrease with age and become insignificant by adolescence (Bouchard, Jr. & McGue, 2003; Deary et al., 2006; Plomin & Spinath, 2004). In concordance, the genetic influences increase, and the heritability of general intelligence may be as high as 80% in adulthood (Posthuma et al., 2002). Longitudinal studies indicate that the stability in general cognitive ability is mainly accounted for by genetic effects, while nonshared environmental effects only exert age-specific influences (Bartels et al., 2002; Bishop et al., 2003; Petrill et al., 2004). Less is known about the development of more specific cognitive abilities. Similar to general intelligence, the heritability of verbal and nonverbal abilities seems to increase with age (Posthuma et al., 2001; Price et al., 2000; Rietveld et al., 2003; Rijsdijk et al., 2002; Wilson, 1986). Longitudinal analyses spanning early to middle childhood suggest that genetic effects are of main importance for the stability in both abilities in this time of development (Cardon, 1994; Rietveld et al., 2003). However, no studies have examined the stability in verbal and nonverbal abilities into later phases of development. Moreover, the developmental structure of the covariance between verbal and nonverbal abilities over time is unclear. Some cross-sectional studies suggest that the overlap between these abilities may increase with age (Posthuma et al., 2001; Price et al., 2000; Rietveld et al., 2003; Rijsdijk et al., 2002; Wilson, 1986). This thesis aims to contribute to the existing literature by studying the development of verbal and nonverbal abilities over a 13-year time span, from early childhood to young adulthood.

Extra attention is devoted to verbal abilities. Verbal abilities are key components for acquiring language, and are needed for healthy social communicative functioning. Although several studies have examined the aetiology of specific verbal measures and components of language and reading at different time points in childhood (e.g. Alarcón et al., 1998; Alarcón et al., 1999; Alarcón et al., 2003; Kovas et al., 2005; Samuelsson et al., 2005), little is known about the overlap between general verbal abilities (as measured for example with the Wechsler verbal IQ scale) and more specialised verbal abilities, such as verbal learning, memory, and fluency. Moreover, it is unclear whether the overlap between these abilities, and the genetic and environ-

mental influences on this overlap, changes over time. In this thesis, the covariance between different verbal abilities is examined in two distinct phases of development: middle childhood and young adulthood.

Milestones in adolescent development: individual differences in testosterone levels and pubertal development

Puberty represents one of the most salient milestones in the development from childhood to adolescence. Secondary sex characteristics emerge, hormonal changes take place, and these changes may be related to developmental changes in cognition and behaviour. Twin studies have shown that pubertal timing is influenced by both genetic and environmental effects, with the estimated influence of genetic factors ranging between 50 to 80% (Eaves et al., 2004; Mustanski et al., 2004; Palmert & Boepple, 2001; Van den Berg et al., 2006). Studies into the aetiology of variance in testosterone levels in males reported heritability estimates ranging from 26 to 66% (Harris et al., 1998; Meikle et al., 1986; Meikle et al., 1988; Ring et al., 2005; Sluyter et al., 2000), with the subjects under study ranging from mid adolescent boys to elderly men. Only one study examined the heritability of testosterone levels in females and found that 41% of the variance in testosterone levels in 14- to 21-year-old women and their mothers was explained by genetic factors (Harris et al., 1998). This thesis aims to explore the heritability of testosterone levels in early puberty. As the variation in sex hormone levels will be related to pubertal maturation at this age, we also included testosterone-related pubertal development in the analyses. Apart from the importance of this topic from a developmental perspective, this paper was also written with a methodological interest. Until recently, methodological constraints prohibited the combined genetic analyses of continuous (such as testosterone levels) and categorical data (such as stage of pubertal development). With the development of the software package Mplus (Muthén & Muthén, 2006), and its application to the twin method (Prescott, 2004), these analyses became possible.

Research design and measures

The greater part of this thesis is based on data collected in a longitudinal twin study into the development of cognition and behavioural problems that was initiated in 1992 with the recruitment of 209 5-year-old twin pairs from the Netherlands Twin Register. The development of these twin pairs was followed at subsequent measurement occasions when the twins were 7, 10, 12, and 18 years of age. At the second to fourth measurement occasion, 92 to 94% of the original sample participated. The last assessment was completed by 122 families from the original sample. At this time point, 64 additional twin families were recruited to obtain a sufficient sample size. Complete data on all 5 measurement occasions were available for 115 twin pairs. The

longitudinal twin sample consisted of 42 monozygotic male twin pairs (MZM), 44 dizygotic male twin pairs (DZM), 47 monozygotic female pairs (MZF), 37 dizygotic female pairs (DZF), and 39 dizygotic twin pairs of opposite sex (DOS). The newly recruited families who only participated at the fifth time point encompassed 13 MZM twin pairs, 12 DZM pairs, 16 MZF pairs, 9 DZF pairs and 14 DOS pairs.

The first to fourth assessments were carried out by three researchers, who all reported on these data in their PhD theses (Bartels, 2003; Rietveld, 2003; Van Baal, 1997). A fifth assessment when the twins were 18 years of age enabled the follow-up of these children into young adulthood. At this time point, siblings of the twins were also included in the study, and collaboration with the Department of Paediatric Endocrinology of the VU medical centre was sought. Medical PhD student Frederiek Estourgie – van Burk collected data on the physical development of the twins and their siblings, while I conducted the psychological test protocol, including the follow-up study of behavioural problems and cognition. In this chapter I will briefly describe the measures that were used for the data analyses in this thesis. Additional information on the longitudinal data collection and on the other measures that were collected as part of the psychological test protocol at the fifth measurement occasion can be found in appendix I.

Autistic traits and behavioural problems

When the twins were 18 years of age, the twins and their siblings filled out the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001). At this time point, all participants also filled out the Dutch Health and Behavior Questionnaire, a large self-report questionnaire including questions about health, wellbeing, leisure activities and behavioural problems (Bartels et al., 2007). Within this questionnaire, problem behaviour was assessed using the Youth Self Report (Achenbach, 1991; Verhulst et al., 1997), a widely used screening and assessment instrument to measure a broad range of behavioural problems.

Cognitive abilities

At all time points, data on psychometric intelligence were collected. At age 5, 7, and 10 years, the twins completed 6 subtests of the Revised Amsterdamse Kinder Intelligentie Test (RAKIT; Bleichrodt et al., 1984). At age 12, the twins completed the Wechsler Intelligence Scale for Children-revised (WISC-R; Van Haassen et al., 1986). At age 18, the twins and their siblings completed 11 subtests of the Wechsler Adult Intelligence Scale-Third edition (WAIS-III; Wechsler, 1997). At all ages, the performance on the intelligence tests could be decomposed into verbal IQ and nonverbal IQ scores. At the fifth measurement occasion, performance on additional verbal tasks was also assessed. Tasks included the California Verbal Learning Test (CVLT; Mulder

et al., 1996) to measure verbal learning and memory, and a test of verbal letter fluency and category fluency. In another twin family project conducted at the NTR (Van Leeuwen et al., 2007), similar data were collected in 9-year-old twins and their siblings. This provided us with the opportunity to compare the aetiology of different verbal abilities in middle childhood and young adulthood.

Hormones and pubertal development

At age 12 and 18 years, the twins (and when the twins were 18 also including the siblings) were asked to fill out a self-report questionnaire assessing their pubertal development, based on the Tanner (Marshall & Tanner, 1969; Marshall & Tanner, 1970) scales. Girls were asked whether they had experienced their menarche, and to rate their stage of breast development and pubic hair growth. Boys were asked about genital development and pubic hair development, and about the size of their testes. The different stages of pubertal development were illustrated by sketches (at the assessment at age 12) or by photographs (assessment age 18). At both measurement occasions, subjects were also asked to collect saliva samples (on 2 consecutive days, just before lunch) for the assessment of biologically active testosterone levels.

Outline of this thesis

This thesis starts with an examination of the reliability and the diagnostic validity of the Dutch translation of the Autism-Spectrum Quotient (Baron-Cohen et al., 2001). Moreover, chapter 2 evaluates the factor structure underlying autistic traits in a large student and general population sample. In chapter 3, the genetic and environmental influences on individual differences in autistic traits are explored in 18-year-old twins and their siblings, and it is tested whether there is evidence for assortative mating for autistic traits in the general population. Chapter 4 discusses an analysis of the genetic and environmental covariation between autistic traits and behavioural problems, as indexed by the Youth Self Report (Achenbach & Rescorla, 2001; Verhulst et al., 1997). The development of withdrawn behavioural problems in childhood is examined in chapter 5, by performing a longitudinal multi-informant twin study into withdrawn behaviour spanning age 3, 7, 10, and 12 years.

Chapter 6 focuses on the stability of verbal and nonverbal intelligence from early childhood to young adulthood. In this chapter, longitudinal IQ data, collected when the twins were 5, 7, 10, 12 and 18 years, are analysed. The genetic and environmental architecture underlying stability in verbal and nonverbal IQ is explored, and it is examined whether the association between these abilities becomes stronger with age. In chapter 7, sources of covariation between verbal IQ and other verbal abilities (that is, verbal learning, verbal memory and verbal fluency) are studied in two independent samples of 9-year-old and 18-year-old twins and their siblings. By including these

two samples, we examined whether the pattern of covariation, and the origins of this overlap, were different in middle childhood and late adolescence. The empirical study of development is concluded in chapter 8, with an examination of the genetic and environmental influences on testosterone levels and its relation to pubertal development when the twins were 12 years of age.

In chapter 9, the results from the preceding chapters are summarised and integrated with the existing literature on these topics. Future directions in the study of autistic traits, withdrawn behavioural problems, and cognitive abilities are considered.

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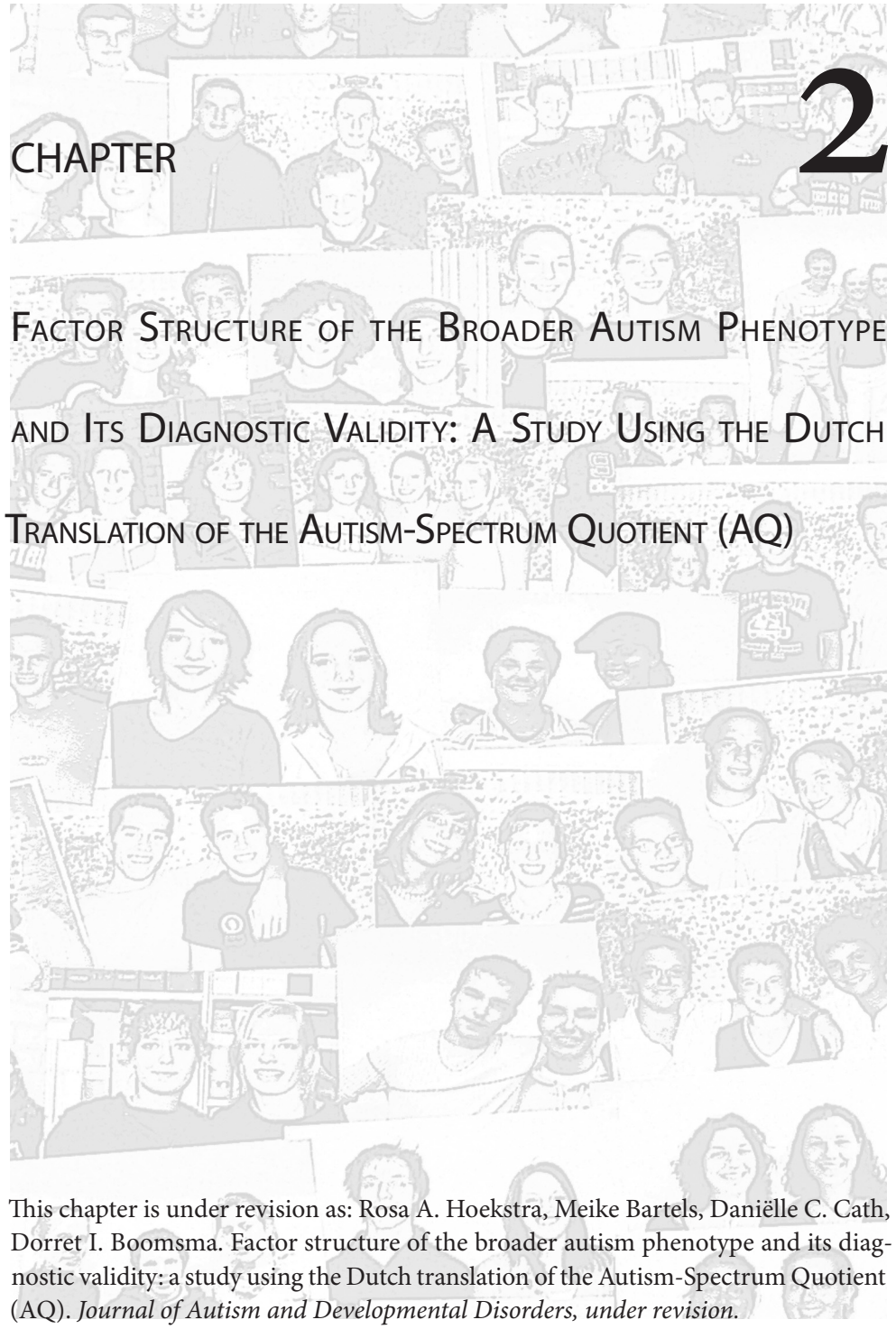

CHAPTER 2

Factor Structure of the Broader Autism Phenotype and Its Diagnostic Validity: A Study Using the Dutch Translation of the Autism-Spectrum Quotient (AQ)

This chapter is under revision as: Rosa A. Hoekstra, Meike Bartels, Daniëlle C. Cath, Dorret I. Boomsma. Factor structure of the broader autism phenotype and its diagnostic validity: a study using the Dutch translation of the Autism-Spectrum Quotient (AQ). *Journal of Autism and Developmental Disorders, under revision.*

Abstract

The factor structure of the Dutch translation of the Autism-Spectrum quotient (AQ; a measure to quantify autistic-like traits) was evaluated with confirmatory factor analyses in a large general population and student sample. The diagnostic validity of the AQ was examined in three matched patient groups (autism spectrum conditions (ASCs), social anxiety, and obsessive-compulsive disorder). A two factor model, consisting of a "social interaction" factor and "attention to detail" factor could be identified. High total AQ and factor scores were specific to ASC subjects. Men scored higher than women and science students higher than non-science students. Internal consistency and test-retest reliability were high. The Dutch translation of the AQ is a reliable instrument to assess the broader autism phenotype.

Keywords: autism; factor analysis; validity; diagnosis; broader autism phenotype

Introduction

Pervasive developmental disorders, of which the most common are autistic disorder, Asperger Syndrome (AS) and pervasive developmental disorder not otherwise specified (PDD-NOS), are characterised by a triad of impairment: difficulties in reciprocal social interaction, communication, and the presence of stereotyped behaviour, interests, and activities (American Psychiatric Association, 2000). Together, these conditions are referred to as autism spectrum conditions (ASCs). This term reflects the assumption that (high functioning) autism and AS lie on a continuum reflecting severity of social communication disability, from classical autism at the most severe end of the spectrum, decreasing via high functioning autism (HFA), AS, and PPD-NOS into normal behaviour. Twin and family studies have shown that genes play a major role in the risk for ASCs (Rutter, 2000). While a conservative estimate of the concordance rates of autism in monozygotic twins is 60%, concordance in dizygotic twins is only 0-5% (Bailey et al., 1995; Folstein & Rutter, 1977). The huge disparity in concordance rates not only shows the strong heritability, but also suggests dominance and epistatic effects involving interaction among genes. Moreover, if one MZ twin has autism, not only is the likelihood of an autism diagnosis in the co-twin increased, but also the risk of other neurodevelopmental difficulties affecting language and social interaction (Bailey et al., 1995; Le Couteur et al., 1996). This notion led to the idea that the same genetic variants affecting the risk for autism may lead to a broader phenotype of autistic traits, as reflected in an increased rate of social deficits, impairments in communication and language, a preference for routines and difficulty with change in non-autistic relatives of autistic individuals (Bailey et al., 1998; Bolton et al., 1994). Rather than a distinct disorder, it is now thought that the autism spectrum conditions as defined in the DSM-IV represent the upper extreme of one or more quantitative traits, and these traits may be continuously distributed in the population (Constantino & Todd, 2003; Piven et al., 1997; Spiker et al., 2002). Studies using quantitative measurements of autistic traits found elevated scores in relatives of autistic and pervasive developmental disorder patients (Bishop et al., 2004; Constantino et al., 2006) and high scores in children whose parents showed high (but sub-diagnosis) endorsement on autistic traits (Constantino & Todd, 2005).

The quantitative approach to autistic traits has led to the development of the Autism-Spectrum Quotient (AQ, Baron-Cohen et al., 2001). This self-administered questionnaire was developed to quantify autistic traits in individuals with normal intelligence. The AQ consists of 50 items, assessing personal preferences and habits. Subjects rate to what extent they agree or disagree with the statements on a 4-point Likert scale, with answer categories "definitely agree"; "slightly agree"; "slightly disa-

gree" and "definitely disagree". For approximately half the items an "agree" response is in line with autistic traits (e.g. item 23: "I notice patterns in things all the time"); for the other half a "disagree" response is indicative of an autistic trait (e.g. item 11: "I find social situations easy"). All the item scores are summed; a high AQ score indicates a high autistic load, close to the autistic end of the autism spectrum. In the original version of the AQ (Baron-Cohen et al., 2001), the 50 items were divided into 5 theoretically derived subscales of 10 items each: social skills; communication; imagination; attention to detail; and attention switching.

Studies using the AQ in England (Baron-Cohen et al., 2001), and Japan (Wakabayashi et al., 2006) found significant higher AQ scores in subjects with an AS or HFA diagnosis, compared to scores in a student sample and a general population sample. Males obtained significantly higher scores than women, and science students scored significantly higher than students in the field of humanities and social sciences. These findings are in line with the 'extreme male brain' theory of autism (Baron-Cohen, 2002). According to this theory, normal sex differences in cognition, skills and interests are more profound in autism, autistic individuals doing extremely well on tasks in which men, on the average, do better than women, and performing poor on tasks in which women typically outperform men. For instance, men, on average, are better in tasks that require systemising (the drive to analyse a system in terms of rules and to predict the behaviour of the system). On the other hand, women are on average better in tasks requiring empathising (the drive to understand another's mental state and appropriately respond to it). If empathising and systemising abilities are seen as two separate continuously distributed dimensions, autistic individuals would fall into the extreme systemising end of the scale, scoring extremely well on systemising tasks, and extremely poor in tasks requiring empathising (Baron-Cohen et al., 2005). Previous studies showed that relatives of autistic individuals more often choose their profession in fields in which high systemising skills are required, such as engineering (Baron-Cohen et al., 1997), or mathematics (Baron-Cohen et al., 2007). Also, students with a parent in scientific occupations score significantly higher on the AQ (Austin, 2005).

Both the British (Baron-Cohen et al., 2001) and the Japanese version (Kurita et al., 2005) of the AQ reported a good test-retest reliability, and a moderate internal consistency (Cronbach's α varying from .63 to .78). However, two aspects of the psychometric properties of the AQ merit further study. Firstly, the five domains of the AQ have been derived theoretically. Performing confirmatory factor analyses can extend our knowledge on the psychometric qualities of the AQ subscales by empirically testing the goodness of fit of the 5 domain model. Austin (2005) conducted an exploratory factor analysis of the AQ in a group of 201 undergraduate students, and found evidence for three, rather than five factors, with a focus on social skills; details/patterns; and communication/mind reading. The first aim of the current study is to examine the model fit of the 5 domain structure proposed by Baron-Cohen et al. (2001) in a large student sample and a general population sample. Secondly, more research needs to be done on the diagnostic validity of the AQ. A short screening instrument for autistic traits is urgently needed since current diagnostic assessment involves lengthy interviews (the most widely used being the Autism Diagnostic Interview-Revised, ADI-R (Lord et al., 1994). It would be a great advantage if only those who are highly likely to have an ASC would have to go through the long diagnostic process, and the AQ could be of help in making this selection. A good screening instrument needs to be both specific (i.e. to be selective to ASC subjects and not to other patient groups or controls) and sensitive (i.e. to recognise ASC subjects as such and keep the number of ASC subjects who "slip through the net" (false negatives) to a minimum). A preliminary study on the AQ (Woodbury-Smith et al., 2005) reported satisfying ability of the AQ to distinguish between subjects with and without an AS/HFA diagnosis, in a group of 100 referrals to a diagnostic clinic for adults suspected of having AS or HFA. To our knowledge, no studies including patients with other psychiatric diagnoses have been reported. The current paper includes a small sample of three different patient groups, one group with AS/HFA and PPD-NOS patients, a group of patients diagnosed with obsessive-compulsive disorder (OCD), and a group with social anxiety disorder (SAD). This way, it can be examined whether a high AQ score is specific to ASCs, or is common to psychiatric disorders in general. Lastly, this paper is the first to examine the psychometric properties of the AQ in a Dutch population. The characteristics of the Dutch AQ, including test-retest reliability and internal consistency, is studied in a large sample of students and subjects from the general population.

Methods

Participants

Four different groups participated in this study. The first group consisted of 965 students from 1) the Vrije Universiteit in Amsterdam (n=813), and 2) the Universiteit Twente in Enschede (n=152). During the break of one of their classes, the students were asked to complete the AQ. Students were recruited from the fields of humanities (history and law; n=129), the social sciences (psychology, education, and communication science; n=597) and natural and technical sciences (including mathematics, physics and information sciences; n=239). Participation rates varied from 65% to 100%. The mean age of the students was 21.19 years (SD=3.69).

The general population sample consisted of parents of twins who visited an information day for parents of multiples. They were asked to either fill out the AQ immediately or to return the questionnaire to our research group by mail. Out of the 500 questionnaires that were handed out, 310 were returned, resulting in a participation rate of 62%. Mean age of the participants was 35.68 years (SD=6.33). The student and general population groups are not matched on age and IQ. However, the two groups are included for separate research purposes. The parent group serves as a normative sample, whereas the student sample is included to address differences in AQ scores in different fields of study.

The third group consisted of three subgroups of psychiatric patients, who were all adult outpatients recruited from the anxiety outpatient services of GGZ Buitenamstel in Amsterdam. All subjects were administered the Structured Clinical Interview on DSM-IV diagnoses (SCID-I; First et al., 1996) to establish in-, and exclusion criteria. Subjects suffering from co-morbid depression, psychosis, substance abuse, mental deficiency or inability to read or speak Dutch were excluded. To exclude any risk of cognitive deficit and/ or below average intelligence, only patients who had successfully completed an educational degree were included in the study, and patient groups were matched on age (range 19 - 57 years), sex (10 males; 2 females in all groups) and educational level. The SCID-I does not contain a section on autism disorders, and at the time of data collection no validated Dutch version of either the Autism Diagnostic Observation Schedule-Generic (ADOS-G, Lord et al., 2000) or the ADI-R (Lord et al., 1994) was available for adult subjects. Therefore all subjects were assessed on presence of ASCs according to DSM-IV criteria by two independent experienced clinicians and with the aid of a structured retrospective interview taken from one of the parents of the patients on early infant development in all domains of the spectrum of autistic conditions. The structured interview encompassed the following topics: age at onset of problem behaviour, contact and communication skills, stereotyped behaviour, development of speech and language, motor and sensory development, particular interests and skills, ability to display imagination, resistance against change and unexpected events, and impulse control. Only subjects who had independently been diagnosed with an ASC by the two clinicians were included in the study; diagnoses were made independent of the AQ responses. Subjects meeting the inclusion criteria completed the AQ at home, after they had given written informed consent, and returned the questionnaire during their next visit to the outpatient service. The patient groups encompassed 1) 12 patients with an autism spectrum condition (n=2 HFA; n=4 AS; n=6 PDD-NOS); 2) 12 patients with a "pure" obsessive-compulsive disorder, 3) 12 patients with a "pure" generalised social anxiety disorder.

To obtain data on test-retest reliability, a group of 18-year-old twins and their brothers and sisters also filled out the AQ. These twin families participate in an ongoing study on cognitive development in late puberty and completing the AQ was part of the test protocol. The first 117 participants of this study were re-contacted one to six months later, and were asked to fill out the AQ for a second time. 75 participants returned the questionnaire for the second time (64%). AQ scores of the responders in the retest did not significantly differ from the subjects who did not respond in the retest ($F(1, 115)=.066$, $p=.797$.

The Dutch Autism-Spectrum Quotient

The AQ was translated after permission from prof. Simon Baron-Cohen (SBC). The translation into Dutch was conducted by an official translator. Subsequently, a second translator translated the Dutch version back into English. After comparing the outcome of the retranslated version to the original text, and discussing discrepancies in the retranslation with SBC, a final version was established (the Dutch version of the questionnaire is obtainable from the first author upon request). Total AQ and domain scores were based on the original 4-point Likert scale scores (1 = "definitely agree" up to 4 = "definitely disagree"). For the items in which an "agree" response is characteristic for autism, the scoring was reversed ("definitely agree" scored 4 points; "slightly agree" 3 points, etc.; This was the case in item 2, 4, 5, 6, 7, 9, 12, 13, 16, 18, 19, 20, 21, 22, 23, 26, 33, 35, 39, 41, 42, 43, 45, 46). All item scores were summed, resulting in a minimum total AQ score of 50 (no autistic traits) and a maximum score of 200 (full endorsement on all autistic items). Note that in the majority of the reports using the British version of the AQ (Baron-Cohen et al., 2001) the answer categories were dichotomised into "agree"/disagree" scores. In these studies, all item responses in line with the autism phenotype scored one point, resulting in a maximum total AQ score of 50.

Missing answers and outliers

If more than 5 items were left blank (10% of the total number of items), the AQ was considered incomplete and the data were discarded in subsequent analyses (n=2 in the student group (in the field of social sciences and humanities); n= 7 in the general population group; none in the patient or twin family groups). Two social sciences students and one subject from the general population group obtained a score > 160. Since these subjects completed the AQ anonymously, it could not be verified whether this reflected a true score. These outliers (deviation > 4 SD's of the mean) were left out in subsequent analyses. This resulted in a final sample size of 961 in the student group and 302 in the general population group.

Statistical analyses

To examine the factor structure of the AQ, models were fitted on the student group data using confirmatory factor analyses in LISREL. To take into account that the items of the questionnaire were measured on an ordinal scale, the diagonally weighted least square procedure was used. Firstly, a one factor solution incorporating all items was fitted. Secondly, a five factor model, based on the 5 domains as suggested by Baron-Cohen et al. (2001), was fitted. The results from this analysis showed high correlations between 4 domains (see results section), and a hierarchical model was fitted to the data, allowing these domains to cluster together. Next, the fit of these models was evaluated in the general population sample, to check whether the same model fits best in a non-student population. Lastly, the best fitting model was applied to the combined student and general population samples (n=1263) to obtain factor loadings and the factor correlation. To evaluate model fit, several model fit statistics were inspected: The χ^2 test statistic; Goodness of Fit Index (GFI); Adjusted Goodness of Fit Index (AGFI); Parsimony Goodness of Fit Index (PGFI); Expected Cross Validation Index (ECVI) and Standardised Root Mean Square Residual (SRMR) (see Schermelleh-Engel et al. (2003) for a comprehensive review of several fit indices and their use).

Group differences in total AQ score and factor scores, and the validity and reliability of the scale were analysed using SPSS. Group differences were tested using mixed model multivariate analysis of variance (MANOVA). The mixed linear model is an expansion of the general linear model and permits non-constant variability in the data. This way, it was possible to test for group differences in variables with unequal variances and unequal group sizes. In the student sample, overall sex differences and differences between students from different fields were explored. Effects of sex and age were studied in the general population sample. Furthermore, AQ score differences between patient groups and the general population sample were tested. To further examine the range of scores in the different patient groups, a frequency distribution was tabulated.

Internal consistency was assessed in the student and general population using Cronbach's alpha. Test-retest reliability was calculated using Pearson's correlation in the 18-year-old twins and their brothers and sisters (n=75). These participants are genetically related. In order to use all available data and to satisfy the independent observations assumption for statistical testing, the test-retest analysis was performed using structural equation modelling in the computer programme Mx (Neale et al., 2006).

Results

Factor analysis

Firstly, a 1 factor model incorporating all 50 factors was fitted using confirmatory factor analysis in the student population. As can be seen in Figure 1, 90% of the items show positive factor loadings in this one factor solution. Subsequently, the 5 domain structure as suggested by Baron-Cohen et al. (2001) was tested. The domains "Social skills"; "Communication"; "Attention switching"; and "Imagination" were highly correlated (correlations varying from r=.53 to r=.84), indicating a considerable overlap between these domains, and suggesting that a model allowing these domains to cluster together may fit the data better. To examine this, a hierarchical model, encompassing 1 higher order factor, existing of 4 lower order domains ("Social skills"; "Communication"; "Attention switching"; and "Imagination") and one separate factor "Attention to detail" was fitted to the data. Fit statistics of the different models

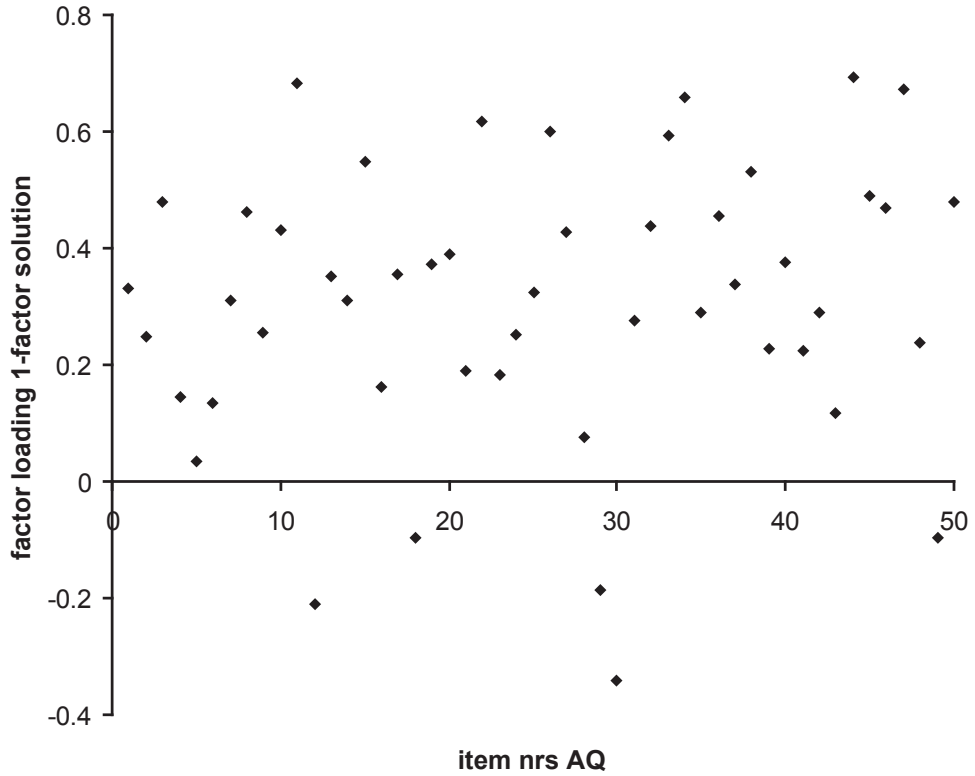

FIGURE 1: Factor loadings of all items of the AQ on the one factor solution in the student population sample. Items are in the same order of appearance as in the questionnaire.

TABLE 1. Summary of the fit statistics of the different factor model structures.

Student sample (N=961)			
	1 factor model (50 items)	5-domain Baron-Cohen et al.	**higher order factor model**
χ^2	14574.788 (p=0.0)	11793.341 (p=0.0)	**11755.716 (p=0.0)**
df	1175	1165	**1170**
ECVI	1.432	1.443	**1.438**
AIC	200.000	220.000	**210.000**
GFI	0.694	0.732	**.730**
AGFI	0.668	0.706	**.705**
PGFI	0.640	0.668	**.670**
SRMR	0.0939	0.0904	**.0907**
General population (n=302)			
	1 factor model (50 items)	5-domain Baron-Cohen et al.	**higher order factor model**
χ^2	5855.664 (p=0.0)	5365.023 (p=0.0)	**5414.112 (p=0.0)**
df	1175	1165	**1170**
ECVI	4.568	4.601	**4.585**
AIC	200.000	220.000	**210.000**
GFI	0.435	0.534	**.525**
AGFI	0.387	0.490	**.483**
PGFI	0.401	0.488	**.482**
SRMR	0.112	0.107	**.107**

Best fitting model is shown bold faced.

are given in Table 1. The hierarchical factor model had the lowest χ^2 value and the highest PGFI (a higher value indicates superior fit) suggesting that this model fitted the data best. The highest values for ECVI, GFI and AGFI (a higher value indicates a better fit) were found in the 5-domain model. However, the differences with the hierarchical model were very small. Since the hierarchical model is a more parsimonious model (df=1170 vs. df=1165), this model was chosen as the best fitting model. Lastly, the fit of all three models was tested in the general population sample. Similar to the student sample, the fit indices were the best for the 5 domain model. However, fit indices for the 5 domain model and the hierarchical model were very similar. Since

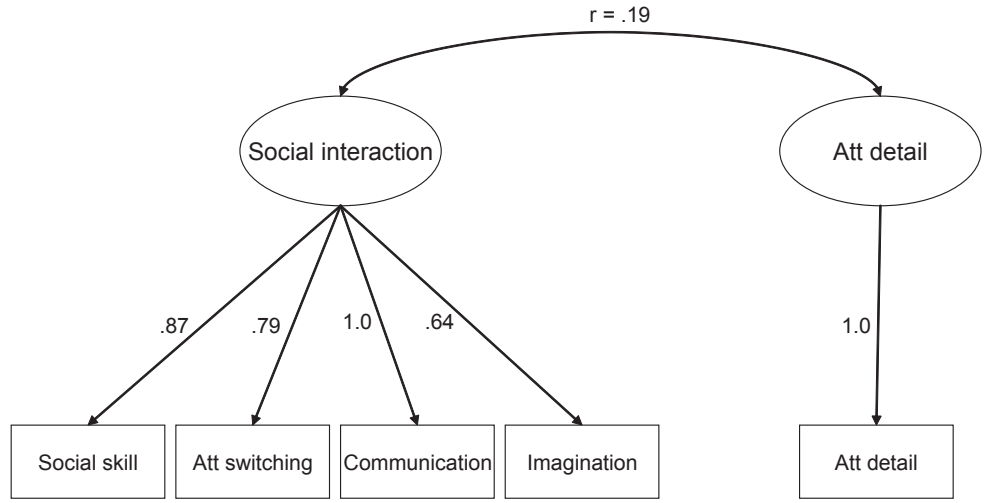

FIGURE 2: Path diagram of the best fitting model with the factor correlation and the standardised estimates of the factor loadings on the underlying domains, as estimated in the combined student and general population sample. Att switching = Attention switching; Att detail = Attention to detail.

the hierarchical model is the more parsimonious model, this model was chosen as the best fitting model.

The hierarchical factor model

The best fitting model (as depicted in Figure 2) consists of one higher order factor, encompassing the 4 lower order domains Social skills; Communication; Attention switching; and Imagination", and one separate factor Attention to detail. Item content and loadings can be found in Table 2. The items loading of the first higher order factor mainly focus on social situations, difficulties in communication with others, and empathic abilities. This factor was called "Social interaction". The second factor is the domain "Attention to detail" and mainly consists of items assessing interests in patterns and details. For both factors, similar to the total AQ score, a high factor sum score implies a high autistic load. The correlation between the 2 factors as assessed in the combined student and general population sample (n=1263) was found to be r=.19 (p<.001).

Internal consistency and test-retest reliability

In the combined student and general population sample, we found the internal consistency of the total AQ score to be satisfactory (Cronbach's alpha=.79,). The internal consistency of the factor scores Social interaction and Attention to detail were

TABLE 2. Item content and loadings on the 5 domains in the best fitting structure, ordered per higher order factors and lower order domains.

Item nr		Domain loading
	Higher order factor Social Interaction	
	Social skill	
1	I prefer to do things with others rather than on my own	.358
11	I find social situations easy	.718
13*	I would rather go to a library than a party	.356
15	I find myself drawn more strongly to people than to things	.537
22*	I find it hard to make new friends	.643
36	I find it easy to work out what someone is thinking or feeling	.450
44	I enjoy social occasions	.748
45*	I find it difficult to work out people's intentions	.499
47	I enjoy meeting new people	.734
48	I am a good diplomat	.232
	Attention switching	
2*	I prefer to do things the same way over and over again	.256
4*	I frequently get strongly absorbed in one thing	.186
10	I can easily keep track of several different people's conversations	.481
16*	I tend to have very strong interests	.172
25	It does not upset me if my daily routine is disturbed	.366
32	I find it easy to do more than one thing at once	.486
34	I enjoy doing things spontaneously	.755
37	If there is an interruption, I can switch back very quickly	.382
43*	I like to plan any activities I participate in carefully	.144
46*	New situations make me anxious	.547
	Communication	
7*	Other people frequently tell me that what I have said is impolite	.279
17	I enjoy social chit-chat	.283
18*	When I talk, it isn't always easy for others to get a word in edgeways	-.116
26*	I don't know how to keep a conversation going	.608
27	I find it easy to "read between the lines"	.419
31	I know how to tell if someone listening to me is getting bored	.290
33*	When I talk on the phone, I am not sure when it's my turn to speak	.560
35*	I am often the last to understand the point of a joke	.293
38	I am good at social chit-chat	.463
39*	People tell me that I keep going on and on about the same thing	.209
	Imagination	
3	Trying to imagine something, I find it easy to create a picture in my mind	.599
8	Reading a story, I can easily imagine what the characters might look like	.576
14	I find making up stories easy	.441
20*	Reading a story, I find it difficult to work out the characters' intentions	.481
21*	I don't particularly enjoy reading fiction	.241
24	I would rather go to the theatre than a museum	.235
40	When younger, I enjoyed playing games involving pretending with other children	.454
41*	I like to collect information about categories of things	.229
42*	I find it difficult to imagine what it would be like to be someone else	.380
50	I find it easy to play games with children that involve pretending	.602

TABLE 2, continued

Item nr		Domain loading
	Factor Attention to detail	
5*	I often notice small sounds when others do not	.252
6*	I usually notice car number plates or similar strings of information	.584
9*	I am fascinated by dates	.754
12*	I tend to notice details that others do not	.148
19*	I am fascinated by numbers	.920
23*	I notice patterns in things all the time	.508
28	I usually concentrate more on the whole picture, rather than the small details	.118
29	I am not very good at remembering phone numbers	.120
30	I don't usually notice small changes in a situation, or a person's appearance	-.135
49	I am not very good at remembering people's date of birth	.120

Note: * designates a reverse-scored item

respectively α=.82 (40 items) and α=.64 (10 items). The test-retest reliability, as assessed in 75 young adults recruited in the twin family study, was .78 for the total AQ score; .77 for the Social interaction factor; and .66 for the Attention to detail factor.

Students: effects of sex and field of study

Table 3 shows the mean total AQ and factor scores in the student sample, separated by field of study. Effects of sex and field of study on the factors Social interaction and Attention to detail score were tested using a mixed model MANOVA. Since these two factors together make up the total AQ score (incorporating all 50 items), sex and field of study effects on the Total AQ score could be tested within the same MANOVA, by simply examining the effect on the sum of the two factors. The MANOVA of Social interaction and Attention to detail by sex, field of study showed a significant main effect of field of study ($F=41.407$, $p<.001$) and a significant sex x field of study interaction effect ($F=5.922$, $p=.003$). Within the different fields of study, the sex effect was not significant ($F=.396$, $p=.530$). The (natural and technical) science students scored significantly higher than the students engaged in a humanities or social sciences degree ($t=8.640$, $p<.001$). The social sciences students scored significantly lower than the humanities and science students ($t=4.482$, $p<.001$). The effect of field of study was significant both in the Social interaction factor ($F=28.215$, $p<.001$) and in the Attention to detail factor ($F=5.336$, $p=.005$).

TABLE 3. Mean total AQ and factor scores per group and sex. Means in the student group separated per field of study.

Group	Sex	N	Total AQ score (SD)	Social interaction factor (SD)	Attention to detail factor (SD)
Humanities students	Total	128[a]	104.46 (12.35)	80.71 (11.88)	23.76 (3.99)
	♂	39	105.54 (12.94)	81.77 (12.91)	23.77 (3.44)
	♀	88	103.99 (12.13)	80.24 (11.43)	23.75 (4.24)
Social sciences students	Total	594[b]	99.07 (11.19)	75.41 (10.09)	23.66 (4.26)
	♂	123	101.32 (12.16)	77.32 (11.17)	24.00 (4.21)
	♀	459	98.47 (10.84)	74.91 (9.73)	23.56 (4.28)
Natural and technical sciences students	Total	239[c]	109.66 (13.37)	85.41 (12.54)	24.26 (4.53)
	♂	203	109.41 (13.73)	85.53 (12.80)	23.89 (4.49)
	♀	32	111.28 (10.81)	84.69 (10.87)	26.59 (4.08)
General population	Total	302[d]	104.20 (11.29)	79.88 (10.68)	24.32 (4.97)
	♂	137	105.66 (10.99)	81.52 (10.98)	24.14 (4.78)
	♀	160	102.93 (11.50)	78.49 (10.25)	24.44 (5.18)
ASC	10♂, 2♀	12	142.25 (22.01)	114.83 (19.12)	27.42 (5.29)
SAD	10♂, 2♀	12	114.17 (16.64)	95.50 (15.01)	18.67 (5.16)
OCD	10♂, 2♀	12	114.83 (12.55)	91.50 (14.07)	23.33 (6.49)

Note: ASC = autism spectrum condition; SAD = social anxiety disorder; OCD = obsessive-compulsive disorder. [a] 1 subject sex unknown; [b] 12 subjects sex unknown; [c] 4 subjects sex unknown; [d] 5 subjects sex unknown. In general population sample: outlier score = 167 left out of the analysis; in student sample: 2 outliers score= 165 and 161 left out of the analysis.

General population: effect of sex and age

Mean total AQ and factor scores in the general population sample are shown in Table 3, separated by sex. A MANOVA of Social interaction and Attention to detail by sex with age showed a significant sex effect (F=5.554, p=.019), men scoring significantly higher than women. The effect of age was not significant (F=.455, p=.500). Contrast tests showed that the sex effect was significant in both the total AQ score

TABLE 4. Frequency distribution of the total AQ score in the general population sample and three patient groups. The ASC group is separated for AS, HFA and PDD-NOS diagnoses.

Total AQ score	General population	ASC	SAD	OCD
50-75	100	100	100	100
76-80	99.7	100	100	100
81-85	97.7	100	100	100
86-90	95.0	100	91.7	100
91-95	89.4	100	91.7	100
96-100	76.8	100	83.3	100
101-105	61.9	100	83.3	83.3
106-110	45.4	100	75	75
111-115	30.1	100 PDD-NOS	50	58.6
116-120	15.9	91.7 PDD-NOS	50	58.3
121-125	7.9	83.3 PDD-NOS	41.7	41.7
126-130	3.3	66.7 PDD-NOS	33.3	25
131-135	1.0	58.3 PDD-NOS	8.3	8.3
136-140	0	50	8.3	8.3
141-145	0	50	8.3	0
146-150	0	50 HFA	0	0
151-155	0	41.7 AS	0	0
156-160	0	25	0	0
161-165	0	25	0	0
166-170	0	25 AS/HFA	0	0
171-175	0	8.3	0	0
176-180	0	8.3	0	0
181-185	0	8.3 AS	0	0
186-200	0	0	0	0

Note: ASC = autism spectrum condition; SAD = social anxiety disorder; OCD = obsessive-compulsive disorder; AS = Asperger syndrome; HFA = high functioning autism; PDD-NOS = pervasive developmental disorder not otherwise specified.

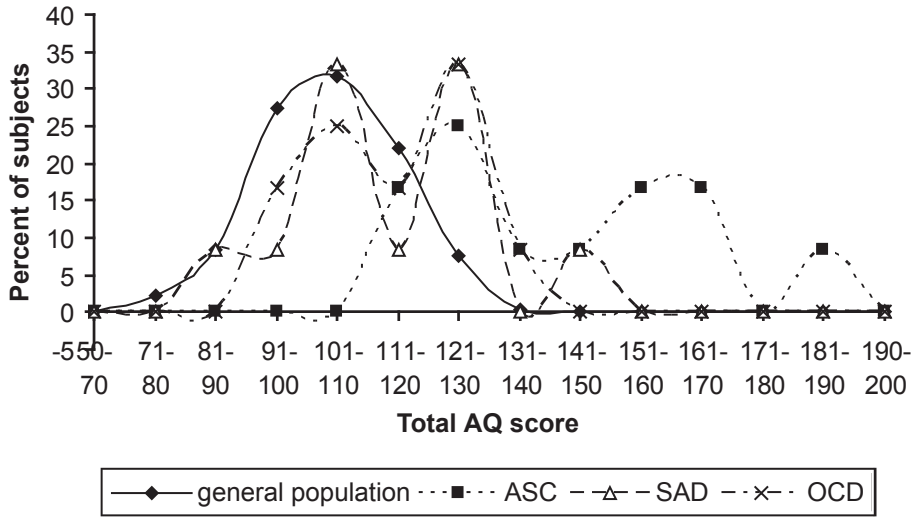

FIGURE 3: AQ scores in the general population and the three patient groups. All subjects scoring >145 were diagnosed with Asperger syndrome or high functioning autism. ASC = autism spectrum condition; SAD = social anxiety disorder; OCD = obsessive-compulsive disorder.

(t=2.006, p=.046) and in the Social interaction factor (t=2.461, p=.014), but not in the Attention to detail factor (t=-.515, p=.607).

Patient groups vs. general population; differences between patient groups

Mean total AQ and factor scores in the different patient groups are shown in Table 3. A mixed model MANOVA of Social interaction and Attention to detail by Diagnosis revealed significant differences between the patient groups and the general population sample (effect diagnosis: F=19.166; p<.001). Contrast tests showed that the subjects diagnosed with an ASC (t=6.894, p<.001), the subjects diagnosed with OCD (t=2.531, p=.022), and the subjects diagnosed with SAD (t=2.243, p=.040) all scored significantly higher on the total AQ score than the general population sample. OCD and SAD patients did not differ in total AQ score (t=-.110, p=.913). The ASC subjects however obtained significantly higher total AQ scores than the OCD (t=3.990, p<.001) and SAD patients (t=3.999, p<.001). Moreover, the ASC subjects scored the highest on the Social interaction factor. Their score on the Social interaction factor was significantly higher than the scores of the general population (t=6.572, p<.001), the OCD sample (t=3.557, p=.002), and the SAD group (t=2.878, p=.009). Lastly, the ASC group scored significantly higher on Attention to detail factor compared to the general population group (t=2.101, p=.036), the OCD patients (t=1.996, p=.047), and the SAD sample (t=4.277, p<.001)

Specificity and sensitivity

The significant differences between patient groups indicate that a high total AQ score is specific to people in the autism spectrum and not for psychiatric patients in general. This observation is confirmed when we take a closer look into the frequency distribution of the total AQ score in the general population, the ASC group, the group of subjects diagnosed with a social anxiety disorder, and the OCD group (Table 4, and Figure 3). A high total AQ score (>145) is specific to ASC subjects, neither subjects from other patient groups, nor subjects from the general population obtain scores in this segment. At a cut off-score of >145, the AQ is highly sensitive to ASC subjects with many autistic traits: all ASC subjects with an AS or HFA diagnosis score in this range. This cut-off score however is not sensitive to ASC subjects with a milder form of autism: Subjects with a PDD-NOS diagnosis obtain scores in the range of 114-134. However, scores in this range (>110) are still higher than the majority of the other psychiatric patients and the general population.

DISCUSSION

The Autism-Spectrum Quotient is a valid and reliable instrument to assess individual differences in autistic-like traits. The Dutch AQ was found to have good psychometric properties, with an internal consistency of α=.79 (similar to the results of Austin, 2005, α=.82 in a British student population), and a test-retest reliability of .78. Confirmatory factor analyses indicated that the original domains (as designed by Baron-Cohen et al., 2001) Social skills, Communication, Attention switching, and Imagination were highly correlated. Rather than four separate domains, we propose a hierarchical model allowing these domains to cluster together. This way, one general "Social interaction" factor (incorporating the four highly correlated domains) could be identified, together with a small second factor, consisting of items focusing on a preference for details and patterns (the domain "Attention to detail"). Previously, an exploratory factor analysis performed in a British student population, suggested three underlying factors within the AQ, encompassing social skills, details/patterns, and communication/ mind reading (Austin, 2005). Our confirmatory factor analysis suggests that, rather than separating the factors social skills and communication/ mind reading, one broader factor assessing (problems with) social interaction fits the data better. Our Social interaction factor not only includes the domains Social skills, Communication, and Imagination, but also the domain Attention switching, which at first thought may seem surprising. As put forward by Courchesne and colleagues (1994), difficulties in attention switching make it harder to keep track of social information. Social interaction usually involves frequent and rapid changes in the source

of information (visually or auditory information, change in objects or actions, etc.), and it requires the ability to follow the flow of social cues (words, gestures, postures, background context, etc). The marked deficit in attention switching in ASC subjects may directly harm their social and communication abilities, which explains why this domain is included within the Social interaction factor.

A small but significant correlation (r=.19) was found between the two factors in our study. This result is in agreement with a British twin project (Ronald et al., 2005) studying both social and non-social behaviours characteristic for autism. They found that both behaviours were highly heritable, but were only weakly correlated to one another (r=.15-.29). The internal consistency of our factors was .82 (Social interaction) and .64 (Attention to detail). The test-retest reliability was satisfactory, with r=.77 for the Social interaction factor, and r=.66 for the Attention to detail factor. Our results indicate that future studies using the AQ to assess autistic traits should focus on the total AQ score and the 2 factor scores, rather than examining differences and similarities in the 5 original domains. The power to detect differences will be higher using the 2 factors; this could be of importance especially in linkage or association studies into autistic traits.

Group differences in AQ scores were in line with previous studies using the AQ (Austin, 2005; Baron-Cohen et al., 2001; Wakabayashi et al., 2006). Males scored higher than females on total AQ score and the Social interaction factor, although no sex difference on the Attention to detail factor was found. Similar to Baron-Cohen et al. (2001) and Austin (2005), science students obtained significantly higher scores than humanities and social science students. In our study, students studying a social science degree in turn scored significantly lower than humanities students. Rather than a remarkable discrepancy with the British results (no differences between humanities and social sciences students), this probably reflects differences in the student sample. In our sample of social science students, "soft" social science degrees like psychology and education were overrepresented. Had "harder" social sciences, such as economics been included, the difference in AQ score might not have been significant.

Subjects diagnosed with an ASC scored significantly higher on the total AQ than the general population and the other patient groups. This is a satisfying result, considering the potential symptom overlap between these patient groups and ASCs. The overlap between ASC subjects and SAD patients entails problems with social interaction in both groups; the overlap between ASC and OCD subjects encompasses repetitive behaviours that occur in both groups. Relatives of autistic individuals are reported to have up to 10-fold higher rates of social phobia compared to control families (Piven & Palmer, 1999; Smalley et al., 1995). Similarly, an increased incidence of OCD is found in autism relatives (Bolton et al., 1998), and the occurrence

of obsessive-compulsive traits in parents of an autistic child is significantly more likely if the child displays strong repetitive behaviour (Hollander et al., 2003). Recent research suggests that a common genetic pathway, the serotonin transporter gene, could explain the association between OCD and rigid compulsive behaviours in autism (Ozaki et al., 2003; Sutcliffe et al., 2005). Our study however shows that high AQ scores are specific to the ASC patients. ASC patients scored significantly higher than the other patient groups on both the Social interaction factor and the Attention to detail factor.

Moreover, all subjects with an AS/HFA diagnosis could successfully be distinguished from the other samples, no subjects without an ASC diagnosis obtained a score >145. However, the difference is less clear-cut for subjects with PDD-NOS. These subjects still obtained higher scores than the majority of the other patients and the normal population, but the specificity was not as good as for the AS/HFA patients. For clinical practice, it could be advised to use two cut-off points: if the aim is to identify subjects with strong autistic characteristics and to keep the number of false positives (i.e. subjects falsely classified in the autism group) to a minimum, it would be advisable to choose >145 as the cut-off point. If the researcher also wants to trace subjects with milder autistic traits (and keep the number of false negatives down) the cut-off point should be decreased to >110. Using a lower cut-off score, further diagnostic testing is needed, since a considerable percentage of subjects with other psychiatric disorders also score within this range.

Two previous studies on the AQ in adults (Baron-Cohen et al., 2001; Woodbury-Smith et al., 2005) have suggested cut-off points for ASC diagnosis. These studies used dichotomised item scores, resulting in total AQ scores between 0 and 50. To examine how our proposed cut-off points compare to the ones suggested by Woodbury-Smith et al. (cut-off point ≥26) and Baron-Cohen et al. (≥32), we reran our analyses using the dichotomised item scores. We found that all AS/HFA subjects scored ≥32 (range 33-48), whilst only one of the OCD patients (range 13-32), one of the SAD patients (range 12-33) and none of the subjects from the normal population (range 5-31) scored above this point. The PDD-NOS subjects in our sample had a total AQ score in the range 24-30, only half of them (3 out of 6) scoring ≥26. It should be noted however, that Woodbury-Smith et al. (2005) chose their cut-off point to optimally distinguish AS patients from non-AS subjects. Their study did not aim to include PDD-NOS diagnoses. The AS subjects in our study all scored above the threshold of ≥26. It should be stressed that our study into the specificity of the AQ are preliminary results. Sample sizes of the patient groups were small. Future studies in clinical samples should more extensively explore the diagnostic validity of the AQ, and should especially focus on the differences between severely impaired ASC patients and mildly impaired PDD-NOS subjects.

In conclusion, this study shows that the AQ is a reliable instrument for screening autistic traits in the general population. Rather than 5 domains, the AQ can be divided into 2 reliable sub factors, focusing on difficulties in social interaction and on marked interests and attention to details and patterns. Results in small patient samples suggest satisfactory discriminative properties of the AQ with other psychiatric disorders, but future studies are needed to further examine this.

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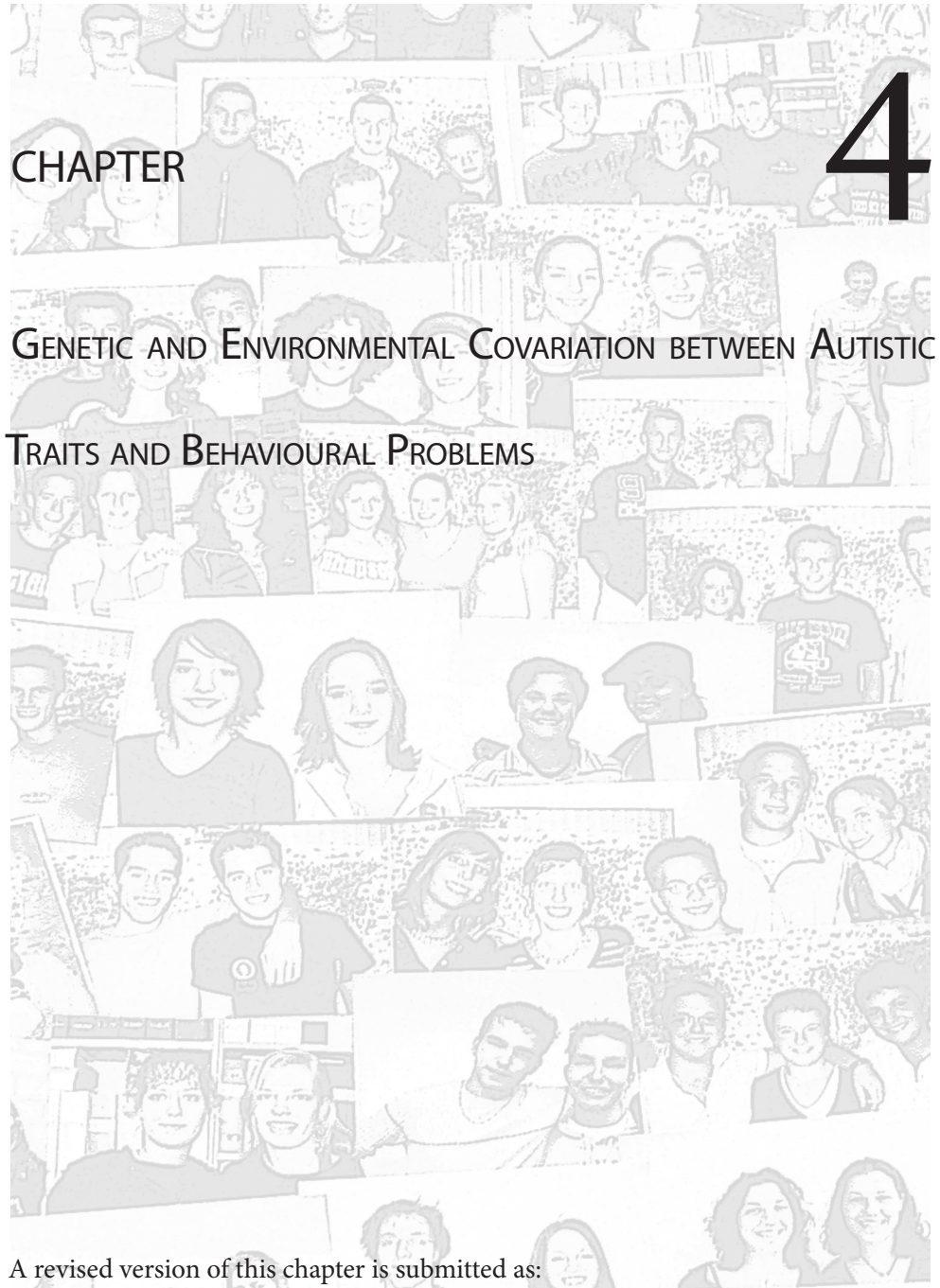

CHAPTER 4

Genetic and Environmental Covariation between Autistic Traits and Behavioural Problems

A revised version of this chapter is submitted as:
Rosa A. Hoekstra, Meike Bartels, James J. Hudziak, Toos (CEM) van Beijsterveldt, Dorret I. Boomsma. Genetic and environmental covariation between autistic traits and behavioral problems. *Genes, Brain and Behavior, submitted.*

Abstract

Objective: To examine the overlap between autistic traits and other behavioural problems in a general population sample, and explore whether this overlap is of genetic or environmental origin.

Method: Youth Self Report (YSR) data were collected in a general population sample of 424 twin pairs at 18 years of age, and their non-twin siblings. In 197 of these families self-report ratings on the Autism-spectrum Quotient (AQ) were collected.

Results: Stepwise backward regression analyses revealed that of all 8 YSR syndrome scales, the Withdrawn Behaviour (WB) and Social Problems (SOC) scale were significant predictors of AQ scores, and together with sex, explained 23% of the variance in AQ scores. Genetic structural equation modelling showed that the overlap between AQ and WB and SOC was mainly due to genetic effects. About half of the genetic variance in AQ scores was specific to the AQ, with the remaining half shared with genetic variance in WB and SOC.

Conclusions: Endorsement of autistic traits in a general population twin family sample is associated with social and withdrawn behavioural problems as measured by Youth Self Reports, and these problems partly share a common genetic aetiology. However, most of the variance in AQ scores remains unexplained by YSR scores, and half of the genetic variance in AQ is unshared with WB and SOC, indicating that autistic traits have specific characteristics that are substantially genetically independent from other common but related behavioural domains such as social problems and withdrawn behaviour.

Key words: autism; autism phenotype; social problems; twins; genetics

Introduction

Autism is one of the most heritable disorders in psychopathology, with the estimated influence of genetic effects above 90% (Freitag, 2007). Twin and family studies have shown that having a relative with autism not only increases the risk for a clinical diagnosis for autism, but also affects the expression of milder but qualitatively similar autistic traits, such as difficulties with social interaction and communication, language deficits, a preference for routines and difficulty with change (Bailey et al., 1998; Bolton et al., 1994; Landa et al., 1992; Piven et al., 1997). These findings have lead to the hypothesis that the same genetic variants that affect the risk for autism may influence the expression of a "broader autism phenotype" in relatives of autistic probands (Piven et al., 1997; Spiker et al., 2002). Rather than treating autism as a distinct disorder, recent studies have used a dimensional approach to study the aetiology of autistic traits (Constantino & Todd, 2000; Constantino & Todd, 2003; Hoekstra et al., 2007b; Ronald et al., 2005; Ronald et al., 2006; Sung et al., 2005). Twin and twin family studies indicate that the strong heritability is not limited to the clinical spectrum, but that genetic effects also account for a moderate to high proportion of the variance in autistic traits in the general population (Constantino & Todd, 2000; Constantino & Todd, 2003; Hoekstra et al., 2007b; Ronald et al., 2005; Ronald et al., 2006).

Individuals with a clinical diagnosis for autism frequently show additional behavioural problems. A systematic review of follow-up studies from childhood to adulthood indicated that depression, often associated with severe anxiety, is the most common psychiatric disorder in high functioning individuals with autism (Howlin, 2000). Other clinical studies suggest that affective disorders are also common in autistic individuals with intellectual disability (Lainhart & Folstein, 1994; Matson & Nebel-Schwalm, 2006). A recent study evaluating the prevalence of psychiatric disorders in children with autism found high prevalence of specific phobia, obsessive compulsive disorder, and attention-deficit hyperactivity disorder (ADHD; Leyfer et al., 2006). Other studies confirm that ADHD-like symptoms, such as inattention, hyperactivity and impulsivity are frequent among children with autism or other pervasive developmental disorders (Goldstein & Schwebach, 2004; Sturm et al., 2004; Yoshida & Uchiyama, 2004). Family studies suggest that relatives of autistic individuals also have increased risk for psychopathology other than autism. Elevated rates of major depression, anxiety, social phobia, and obsessive compulsive disorders are reported in relatives of children with autism (Bolton et al., 1998; Micali et al., 2004; Piven & Palmer, 1999; Smalley et al., 1995). However, it remains unclear whether these elevated risks can be attributed to the burden of caring for an autistic child, or may be due to genetic risk factors associated to the risk for autism. The pattern of

familial aggregation of OCD and affective disorders in a family study by Bolton et al. (1998) suggested that OCD, but not affective disorders may underlie a genetic liability for autism.

The dimensional approach to autistic traits opens a window of opportunities to study the aetiology of the overlap between autistic symptoms and other domains of psychopathology in general population samples. One previous study examined the overlap between deficits in social reciprocal behaviour and other behavioural problems in a sample of 7- to 15-year-old male twins (Constantino et al., 2003). Genetic and environmental influences on covariation between scores on the Social Responsiveness Scale (SRS) and syndrome scores of the Child Behavior Checklist (CBCL) were examined. Multiple regression analysis indicated that CBCL syndromes accounted for 43% of the variance in SRS scores, with the largest contribution coming from the syndrome scales Attention Problems and Social Problems. The variance in SRS scores was explained by phenotypic effects shared with the Social Problems score, by nonshared environmental effects, and by genetic effects specific to the SRS. This study only included male twins, and the SRS and CBCL scores were not collected simultaneously, but up to two years apart. So far, no studies have examined the overlap between autistic traits and other behavioural problems in late adolescence, and none have included females and non-twin siblings. The current study wishes to address these issues. The aim of this study is to examine the overlap between quantitative autistic traits, as measured with the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001; Hoekstra et al., 2007b) and behavioural problems (as indexed by Youth Self Report scores (YSR; Achenbach & Rescorla, 2001; Verhulst et al., 1997), in a general population sample of 18-year-old twins and their siblings. The AQ and YSR data were collected in the same time period. The genetic nature of this study enables to distinguish genetic and environmental influences on the overlap between these domains of problem behaviour.

Methods

Participants and procedures

All participants were contacted via the Netherlands Twin Register (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam (Bartels et al., 2007; Boomsma et al., 2006). From 1986 onwards, the NTR has recruited families with multiples a few weeks or months after birth. When the twins are 1, 2, 3, 5, 7, 10, and 12 years old, the parents are asked to provide information about the physical and behavioural development of their twins via mailed surveys. At age 14, 16, and 18 parents are asked for permission to contact the twins and additional siblings

in the family. These offspring are invited to register with the NTR and are asked to fill out self-report questionnaires. The current study includes data from twins born in 1986 – 1988 (mean age=18.44, sd=.39), and their siblings (mean age 19.00, sd=4.19). Youth Self Report data were collected in 424 families. These included 65 monozygotic male pairs (MZM), 60 pairs were dizygotic males (DZM), 106 were monozygotic females (MZF), 88 were dizygotic females (DZF), and 105 were dizygotic twin pairs of opposite sex (DOS). Data of an additional sibling were available for approximately half of the families (206 siblings, of which 96 were male, 110 were female). Zygosity of the same sex twin pairs was determined using DNA analysis (178 pairs), blood group polymorphisms (48 pairs) or longitudinally collected questionnaire items (Rietveld et al., 2000; 93 pairs). In a subset of this sample (197 families), AQ data were collected. These twin families participated in a longitudinal study into the development of cognition and problem behaviour, and encompassed 37 MZM, 34 DZM, 47 MZF, 40 DZF, and 39 DOS twin pairs, and 104 siblings (52 brothers and 52 sisters). All subjects filled out the YSR at home. Most twin families from the subset (n=186) filled out the AQ at the VU University as part of an extensive test protocol. Eleven families filled out the AQ at home (AQ scores in these families were not different from the participants who visited the VU University). Complete data on both the YSR and the AQ were available for 452 subjects (all from the subset of 197 families).

Measures

The AQ is a self-administered questionnaire developed to quantify autistic traits in individuals with normal intelligence (Baron-Cohen et al., 2001). The AQ consists of 50 items, selected from the domains of the "triad of impairment" in autism (impaired social skills, impaired communication, and restricted interests), and from demonstrated areas of cognitive abnormality in autism (e.g. lack of imagination and great attention to detail). Participants rate to what extent they agree or disagree with the statements on a 4-point Likert scale, with answer categories "1 = definitely agree"; "2 = slightly agree"; "3 = slightly disagree" and "4 = definitely disagree". For items in which an "agree" response is characteristic for autism (24 out of the 50 items), the scoring was reversed. AQ scores were calculated as the sum scores, with a minimum AQ score (50) indicating no autistic traits, and a maximum score (200) indicating full endorsement of all autistic traits. The Dutch translation of the AQ has good internal consistency (Cronbach's alpha=.79) and test-retest reliability (r=.78 in a group of 75 subjects with a 1-6 month time interval; Hoekstra et al., 2007a). Complete AQ's were available for 470 subjects. If 5 or less answers were missing, the AQ score was corrected for the number of missing items by adding the mean item score times the number of missing items to the total AQ score (1 missing answer n=22; 2 missing answers n=3).

The YSR is a self-report questionnaire designed to assess emotional and behavioural problems in 11- to 18-year-old children (Achenbach & Rescorla, 2001; Verhulst et al., 1997). The adolescent is asked about the occurrence of problem behaviour in the preceding 6 months and to rate the behaviour on a 3-point scale (0 if the problem item is not true; 1 if the item is somewhat or sometimes true; and 2 if it is very true or often true). The YSR generates scores for 8 syndrome scales: Anxious/Depressed, Withdrawn Behaviour, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Aggressive Behaviour, and Rule-Breaking Behaviour. Similar to the original version (Achenbach & Rescorla, 2001) the Dutch version of the YSR shows good reliability and validity (Verhulst et al., 1997).

Data analysis

To examine the extent to which the 8 YSR syndrome scales predicted AQ scores, multiple regression analysis was conducted. Since the inclusion of twin and twin-sibling data violated the assumption of independent observations, the regression analysis was carried out using structural equation modelling in Mx (Neale et al., 2006), by allowing the data from family members to be correlated. The YSR syndrome scales were included in the regression analysis as definition variables. As previous studies showed significant sex differences in mean AQ scores (Baron-Cohen et al., 2001; Hoekstra et al., 2007b; Wakabayashi et al., 2006), sex was added as an additional predictor. A procedure similar to the backward stepwise regression method was employed. Firstly, all predictors were entered in the regression equation. Next, the predictor explaining the least variance in AQ scores was dropped from the model. This procedure was repeated until the significance of each YSR syndrome scale was tested. Those YSR scales that were found to be significant predictors of AQ scores were subsequently included in the multivariate genetic analyses.

To assess the degree of covariation between AQ scores and YSR syndrome scales, and the extent to which the selected YSR syndrome scales correlated with each other, phenotypic correlations were estimated in a saturated model in Mx. The saturated model was also used to estimate twin and twin-sibling correlations for each variable, and to estimate the twin and twin-sibling cross-correlations (e.g. the correlation between the AQ score of the oldest of the twin and the YSR syndrome score of the youngest of the twin). All data were used, including YSR data of subjects without information on the AQ and including data from families for whom information of one of the twins was missing, or for whom no sibling data were available.

Genetic analyses

Since monozygotic (MZ) twins are genetically identical at the DNA sequence level, while dizygotic (DZ) twins and non-twin siblings share on average 50% of their

segregating genes, genetic model fitting of twin family data allows for separation of the observed phenotypic variance into its genetic and environmental components. Additive genetic variance (A) is the variance that results from the additive effects of alleles at each contributing gene locus. Shared environmental variance (C) is the variance due to environmental factors shared between the offspring, nonshared environmental variance (E) is the variance resulting from environmental effects unique to each family member and also includes measurement error.

The twin correlations and twin-sibling correlations provide a first impression of the genetic and environmental contributions to the variance. A stronger resemblance between MZ twins than between DZ twins and twin-siblings indicates that genetic effects are important. Similar correlations between MZ twins and first-degree relatives suggest that shared environmental influences could play a role. A less-than-perfect correlation between MZ twins indicates influence of the nonshared environment. Multivariate genetic analysis provides insight in the aetiology of the covariance between traits. Higher cross-correlations in MZ twins compared to DZ twins and twin-siblings indicate genetic influences on the overlap between traits, while similar cross-correlations in MZ twins and first-degree relatives suggest shared environmental influences.

Genetic model fitting was performed in Mx using a Cholesky or triangular decomposition (Neale & Cardon, 1992) in which the observed variance-covariance matrix is decomposed into genetic, shared environmental, and nonshared environmental matrices. This decomposition was used as a reference model to evaluate the fit of more restricted models. The significance of sex differences in the variance components was tested by examining the deterioration in model fit after constraining the magnitude of A, C, and E to be equal across the sexes. The significance of the contribution of A and C was tested by assessing the decrease in model fit after each component was dropped from the model. The fit of the various models was compared using likelihood ratio tests. The likelihood ratio is the difference between the minus two log likelihoods (-2LL) under two nested models and follows a χ^2 distribution. The degrees of freedom (df) are given as the difference in the number of estimated parameters in the two models. A high increase in χ^2 against a low gain of df denotes a worse fit of the submodel compared to the full model.

Results

The AQ scores were continuously distributed. Males obtained significantly higher scores than females (χ^2=19.713, df=1, p<.001). Multiple regression analysis (see Table 1) revealed that the YSR syndrome scales Withdrawn Behaviour (WB) and

TABLE 1. Multiple linear regression results: associations between AQ scores and YSR syndrome scales.

test	model	-2LL	df	cpm	χ^2	df	p
1	Model including all YSR scales + sex	3139.559	422				
2	Drop YSR ATT	3139.561	423	1	0.002	1	.963
3	Drop YSR ANX	3139.587	424	2	.026	1	.872
4	Drop YSR AGG	3139.642	425	3	.055	1	.815
5	Drop YSR SOM	3141.921	426	4	2.279	1	.131
6	Drop YSR RB	3148.157	427	5	6.236	1	.013
7	Drop YSR THO	3153.701	428	6	5.544	1	.019
8	Drop YSR SOC	3170.964	429	7	17.263	1	<.001
9	Drop YSR WB	3249.176	430	8	78.212	1	<.001

Note: -2LL = -2 log likelihood; cpm = compared to model; YSR ATT = YSR syndrome scale Attention Problems; ANX = Anxious/Depressed; AGG = Aggressive Behaviour; SOM = Somatic Complaints; RB = Rule-Breaking Behaviour; THO = Thought Problems; SOC = Social Problems; WB = Withdrawn Behaviour.

Social Problems (SOC) were both significant predictors of AQ scores (respectively χ^2=78.212, df=1, p<.001, and χ^2=17.263, df=1, p<.001). To ensure that we would select YSR syndrome scales with a robust predictive effect, explaining a meaningful proportion of the variance in AQ scores, we used a conservative p-value of p<.01. Keeping this p-value, the remaining syndrome scales failed to be significant. To check for possible effects of influential data points, we created scatter plots of all AQ scores against the YSR syndrome scores and detected 6 outliers. We ran the regression analysis again without these data points and found similar results for the effect of sex (χ^2=17.000, df=1, p<.001), WB (χ^2=75.3=232, df=1, p<.001), and SOC (χ^2=15.694, df=1,

TABLE 2. Sample sizes, means, and standard deviations for scores on the AQ, YSR Withdrawn Behaviour (WB), and YSR Social Problems (SOC).

	N	Mean	SD
AQ male	218	103.99	10.48
AQ female	252	100.69	10.61
AQ all[a]	470	102.22	10.67
WB male	397	2.36	2.15
WB female	569	2.67	2.17
WB all[bc]	966	2.54	2.16
SOC male	398	1.91	1.74
SOC female	570	1.88	1.72
SOC all	968	1.90	1.72

[a]sex effect significant p<.01; [b]sex effect significant p<.05; [c]age effect significant p<.01

p<.001). The predictive effect of the YSR scale Rule-Breaking Behaviour however was strongly attenuated (χ^2=2.152, df=1, p=.142), the effect of YSR Thought Problems also decreased slightly (χ^2=5.090, df=1, p=.024). All in all, WB and SOC were found to explain a significant proportion of the variance in AQ scores in both analyses. The regression model including these two YSR scales and sex as predictors explained 23% of the observed variance in AQ scores (i.e. R^2=23%), of which 21% was due to the YSR scores. Stein's adjusted R^2 for the regression model (Stevens, 1996) was 21.7%. This value is very similar to the observed value of R^2, indicating that the cross-validity of this model is good.

The descriptive statistics of AQ scores, WB and SOC are provided in Table 2. Apart from the sex effect on mean AQ scores, a significant age (χ^2=7.589, df=1, p=.006) and sex effect (χ^2=4.870, df=1, p=.027) was found for mean WB scores, with higher scores in females than males, and increasing WB scores with age. In subsequent modelling, the means were corrected for these effects. The variances (χ^2=1.201, df=3, p=.753) and within person covariance (χ^2=1.521, df=3, p=.677) could be equalled in male and female twins and across all twins and siblings (respectively χ^2=1.145, df=3, p=.766 and χ^2=5.203, df=3, p=.158), indicating that the phenotypic correlations between the measures are similar in males and females and in twins and their siblings. The phenotypic correlations between AQ scores and WB and SOC scores (first row of Table 3) are moderate, with WB explaining 20% ($.45^2$) of the variance in AQ scores, and SOC explaining 16% of the variance in AQ scores. The two YSR syndrome scales are

TABLE 3. Phenotypic correlation in all participants, and twin and twin-sibling correlations and cross-correlations for AQ, YSR Withdrawn Behaviour and Social Problems in all zygosity groups and twin-sibling pairs (MZM, DZM, male twin-sibling, opposite sex twin-sibling, all DZ, all 1st degree relatives above diagonals; MZF, DZF, female twin-sibling, DOS, and all MZ below diagonals).

	AQ	WB	SOC
		Phenotypic	
AQ	-	.45	.40
WB		-	.53
SOC			-
		MZF/MZM	
AQ	.41/.60[a]	.31	.41
WB	.23	.59/.47[a]	.26
SOC	.26	.38	.52/.38[a]
		DZF/DZM	
AQ	.45/.37[b]	.09	.17
WB	.23	.27/.28[b]	.16
SOC	.16	.17	.31/.30[b]
		Female twin-sibling/Male twin-sibling	
AQ	.42/.10[c]	.18	.16
WB	.21	.10/.17[c]	.13
SOC	.31	.18	.19/.05[c]
		DOS/Opposite sex twin-sibling	
AQ	.36/.25[d]	.07	.11
WB	.16	.29/.09[d]	-.04
SOC	.14	.25	.24/.00[d]
		All MZ/ All DZ	
AQ	.49/.39[e]	.16	.17
WB	.27	.55/.28[e]	.20
SOC	.33	.34	.46/.29[e]
		All 1st degree relatives	
AQ	.33	.17	.19
WB		.17	.11
SOC			.14

Note: MZM = monozygotic males; DZM = dizygotic males; MZF = monozygotic females; DZF = dizygotic females; DOS = dizygotic twins of opposite sex.
[a]First figure correlation MZF, second figure correlation MZM; [b]correlation DZF/DZM; [c]correlation female twin-sibling pairs/male twin-sibling pairs; [d]correlation DOS/opposite sex twin-sibling pairs; [e]correlation all MZ/all DZ.

TABLE 4. Model fitting results for multivariate analyses of AQ scores, YSR Withdrawn Behaviour, and Social Problems.

test	model	-2LL	df	cpm	χ^2	df	p
1	ACE sex differences	11005.799	2362				
2	ACE no sex differences	11024.243	2380	1	18.444	18	.427
3	ACE AQ, ACE WB, AE SOC, no sex differences	11024.669	2383	2	.426	3	.935
4	ACE AQ, AE WB, AE SOC, no sex differences	11025.041	2385	3	.372	2	.830
5	**AE no sex differences**	11025.765	2386	4	.724	1	.394
6	CE AQ, ACE WB, ACE SOC, no sex differences	11032.265	2381	2	8.022	1	.004
7	ACE AQ, CE WB, ACE SOC, no sex differences	11045.508	2382	2	21.265	2	<.001
8	ACE AQ, ACE WB, CE SOC, no sex differences	11041.222	2383	2	16.979	3	<.001

Note: -2LL = -2 log likelihood; cpm = compared to model; A = additive genetic influences; C = shared environmental influences; E = nonshared environmental influences; WB = YSR Withdrawn Behaviour; SOC = YSR Social Problems.

substantially correlated with each other (r=.53).

On the diagonal of Table 3, the twin correlations are presented for all zygosity groups and twin-sibling combinations. On the whole, the correlations in MZ twins are higher than in DZ twins and twin-siblings, indicating genetic influences. The MZ twin correlations for AQ scores are not twice as high as the correlations in first-degree relatives, suggesting that shared environmental influences could also play a role. The MZ twin cross-correlations (off-diagonal of Table 3) are higher than the cross-correlations in first-degree relatives, suggesting that the overlap between AQ, WB and SOC is influenced by genetic factors. The MZ twin cross-correlations be-

tween AQ and the two YSR scores are not twice as high as the cross-correlations in first-degree relatives, suggesting that shared environmental influences may explain part of the phenotypic overlap between these measures.

Table 4 presents the model fitting statistics for the full Cholesky model, including both additive genetic, shared environmental, and nonshared environmental influences (referred to as the ACE model), and for the more parsimonious submodels. Constraining the parameters that represent the effect of A, C, and E to be the same in males and females did not significantly worsen the model fit (model 2, χ^2=18.444, df=18, p=.427), suggesting that the relative effects of these components are the same across the sexes. The shared environmental component could be dropped from the model without a significant deterioration of the fit (models 3 to 5). The genetic effects however were of significant importance for all measures (models 6 to 8). The best fitting most parsimonious model was an AE model without sex differences in the relative contribution of the variance components (model 5).

The contributions of additive genetic and nonshared environmental effects to the variance in AQ, WB and SOC are shown on the diagonal of Table 5. Genetic effects explain 54% of the individual differences in AQ scores, the remaining variance is accounted for by nonshared environmental effects. Genetic influences explain about half of the variance in WB, and account for 41% of the variance in SOC. The overlap between AQ scores and scores on the YSR scales (off-diagonal of Table 5) is largely due to genetic effects, explaining 64% of the covariance between AQ and WB, and 82% of the covariance between AQ and SOC. The remaining covariance between the

TABLE 5. Contributions of additive genetic (A) and nonshared environmental (E) effects to the variance and covariance in AQ scores and in YSR Withdrawn Behaviour (WB) and Social Problems (SOC) scores. Estimates are based on the best fitting model (95% confidence interval in parentheses).

	A			E		
	AQ	WB	SOC	AQ	WB	SOC
AQ	.53 (.39-.64)			.47 (.36-.61)		
WB	.64 (.41-.84)	.50 (.38-.60)		.36 (.16-.59)	.50 (.40-.62)	
SOC	.82 (.58-1.03)	.60 (.44-.75)	.41 (.29-.52)	.18 (-.03-.42)	.40 (.25-.56)	.59 (.48-.71)

measures is accounted for by nonshared environmental influences. Examination of the individual parameter estimates showed that 51% of the genetic variance in AQ is shared with the variance in YSR scores, the remaining genetic variance is specific to the AQ. Only 11% of the nonshared environmental variance is shared with variance in YSR scores, implying that the greater part (89%) of this variance is specific to the AQ.

Discussion

This study reported on the overlap between autistic traits and behavioural problems in a general population sample in late adolescence. The YSR syndrome scales WB and SOC were found to be significant predictors of endorsement of autistic traits, and could, together with sex as additional variable, explain 23% of the variance in AQ scores. Genetic analyses showed that individual differences in AQ scores underlie substantial genetic influence. Moreover, the overlap between AQ, WB and SOC scores was mainly accounted for by genetic effects. Approximately half of the genetic variance in AQ scores was shared with variance in the YSR scales, the nonshared environmental variance in AQ was largely specific to the AQ.

The finding that the YSR scales Withdrawn Behaviour and Social Problems were the best predictors of AQ scores is not surprising. The WB syndrome scale captures shy, introvert and withdrawn behaviour, and includes items such as "Prefer to be alone"; "Secretive" and "Withdrawn". The SOC scale contains items such as "Too dependent"; "Teased a lot"; and "Other children don't like me". These items all hint at difficulties in social interactions, one of the main impairments in autism, and similar items (such as "I prefer to do things with others rather than on my own" and "I find it hard to make new friends") are also included in the AQ. However, the WB scale also includes items ("Lacks energy" and "Sad") more indicative of depression. Examination of the raw item scores revealed that these items loaded as strong on the AQ scores as the other items of the WB scale. The syndrome scales WB and SOC are substantially correlated with the Anxious/Depressed scale of the YSR, both in our sample (r=.66 for WB, and r=.49 for SOC), and in the general population sample from which the Dutch YSR norm scores were derived (r between .46 and.62; Verhulst et al., 1997). The AQ scores in our sample correlated moderately with the Anxious/Depressed scale (r=.31), but this association failed to be significant once the association with WB and SOC was taken into account. Altogether, these results suggest that social problems related to anxiety and depression may be common in people highly endorsed on autistic traits.

In a study into the overlap between reciprocal social behaviour deficits and behavioural problems in 7- to15-year-old male twins (Constantino et al., 2003), the SOC scale of the CBCL was found to account for a significant proportion of the variance in SRS scores. The WB scale was the third most important predictor, but failed to be significant after Bonferroni correction for multiple testing. In contrast to our study, the Attention Problems scale was found to account for a significant proportion of the variance. This discrepancy could be due to a variety of factors. Firstly, the study by Constantino and colleagues only included males and was performed in younger twins, aged 7-15 years. The association between autistic-like behaviours and attention problems may be stronger in this earlier phase of development. Furthermore, differences in the questionnaires used to assess autistic traits (parent-report SRS vs self-report AQ) may underlie the different findings. The SRS inquires about problem behaviours directly, while the AQ assesses personal preferences and habits, rather than a judgment of behaviour. In a clinical sample of children with autism, parent-reported CBCL syndrome scores were evaluated, and the highest relative scores were found on Attention Problems, Social Problems, and Thought Problems (Bolte et al., 1999). Another study compared parent-reported CBCL scores in children with problems classified as pervasive developmental disorder not otherwise specified (PDD-NOS), children classified as ADHD, and a group of normal functioning controls (Luteijn et al., 2000). Both the PDD-NOS and the ADHD group showed elevated scores on the Attention Problems scale, these groups did not differ from each other. High scores on the WB and SOC scale were specific to the PDD-NOS group. Our study suggests that in the general population, the observed overlap between autistic traits and withdrawn and social behavioural problems is primarily of genetic origin.

This study has some limitations. The sample size for which both data on the AQ and the YSR were available was relatively small. Therefore, the power to detect sex differences in the genetic and environmental architecture underlying the association between the AQ and the YSR scores was limited. Future studies should include larger sample sizes to further examine this issue. Another limitation is the exclusive reliance on self-report measures of problem behaviours. Previous studies have shown that autistic traits and behavioural problems can reliably be assessed using self-report AQ (Baron-Cohen et al., 2001; Hoekstra et al., 2007a) and YSR data (Verhulst et al., 1997). However, previous studies also indicate that different raters can provide important additional information (Bartels et al., 2003; Ronald et al., 2005; Van der Ende & Verhulst, 2005). Multiple informant data should be collected in future studies. It should be stressed that, as yet, it remains unknown whether the genetic influences on autistic traits in the general population are the same as those affecting the risk for clinical autism. Likewise, it is unknown whether the gene variants influencing the association between autistic traits and withdrawn and social behavioural problems

will be the same in a clinical sample. Future research in genetically informed clinical samples is needed to clarify this issue.

Our study suggests that the vulnerability for general social problems is genetically related to endorsement of autistic traits. About half of the genetic variance in AQ scores was shared with genetic variance in WB and SOC scores. However, this also indicates that half of the genetic variance is specific to the AQ and thus unshared with other behavioural problems. This result supports the findings of Constantino et al. who, when examining the overlap between SRS and CBCL scores, concluded that autistic traits (or, in their study: deficits in social reciprocal behaviour) constitute a genetically independent domain in psychopathology (Constantino et al., 2003). In our study the WB and SOC scores only explained 23% of the variance in AQ scores, signifying that the bulk of variance in AQ scores remains unexplained, and suggesting that the YSR on the whole is not ideal to trace behavioural problems indicative of autism. The YSR subscales WB and SOC however could be used as predictors for endorsement of autistic traits.

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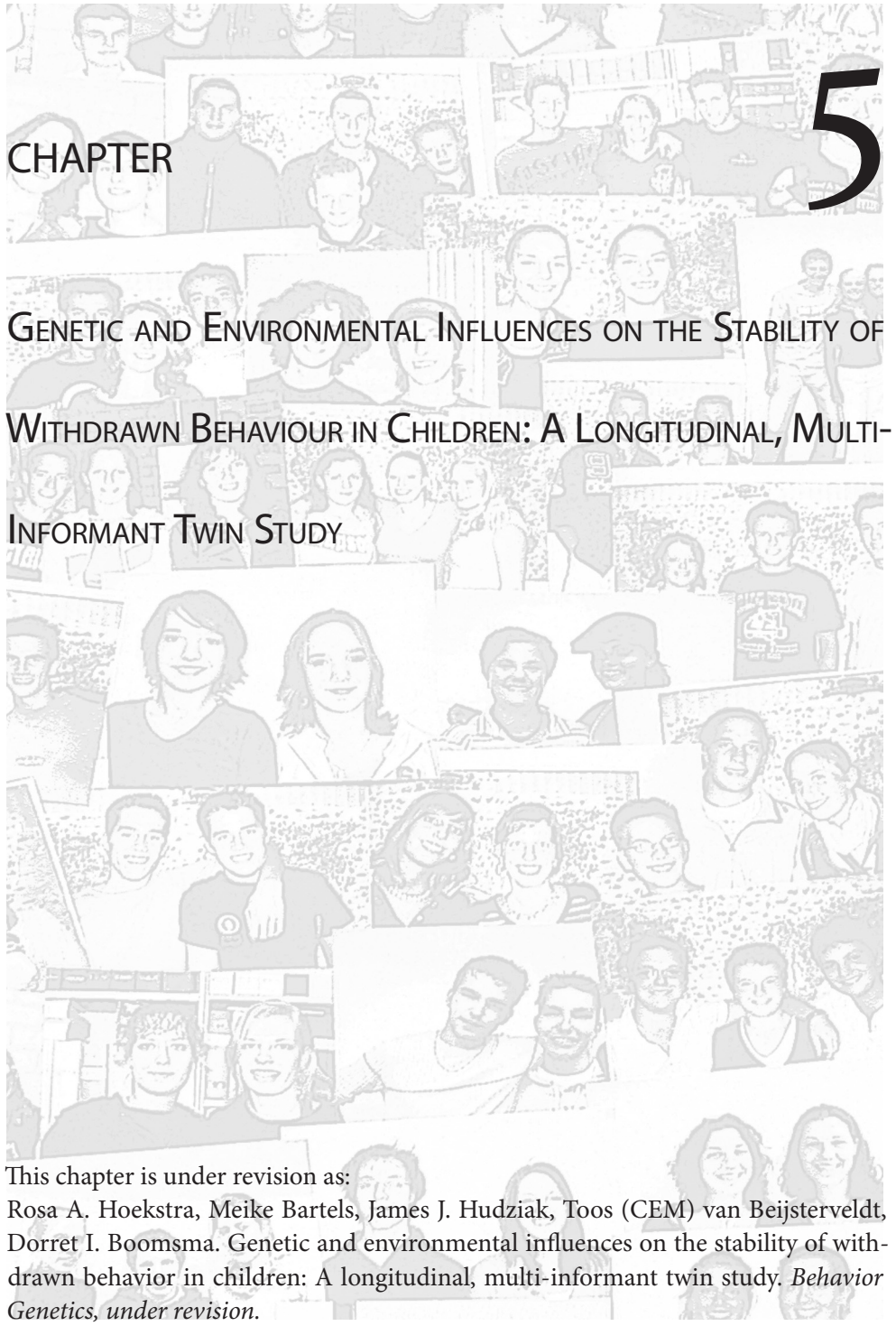

CHAPTER 5

Genetic and Environmental Influences on the Stability of Withdrawn Behaviour in Children: A Longitudinal, Multi-Informant Twin Study

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Rosa A. Hoekstra, Meike Bartels, James J. Hudziak, Toos (CEM) van Beijsterveldt, Dorret I. Boomsma. Genetic and environmental influences on the stability of withdrawn behavior in children: A longitudinal, multi-informant twin study. *Behavior Genetics, under revision.*

Abstract

This study examined the contribution of genetic and environmental influences on the stability of withdrawn behaviour in childhood using a longitudinal multiple rater twin design. Maternal and paternal ratings on the withdrawn subscale of the Child Behavior Checklist were obtained from 14889 families when the twins were 3, 7, 10 and 12 years old. Withdrawn behaviour showed considerable stability throughout childhood, with correlation coefficients ranging from about .30 for the 9-year time interval to .65 for shorter time intervals. Individual differences in withdrawn behaviour were found to be largely influenced by genetic effects at all four time points, in both boys (50-66%) and girls (40-61%). Shared environmental influences explained a small to modest proportion (2 – 23%) of the variance at all ages and were slightly more pronounced in girls. Nonshared environmental influences were of moderate importance to the variance at all ages in both boys (21-38%) and girls (24-41%).The stability of withdrawn behaviour was largely explained by genetic effects, accounting for 74% of stability in boys and 65% in girls. Shared environmental effects explained 8% (boys) and 18% (girls) of the behavioural stability. As the main part of the shared environmental influences on stability was common to both raters, these effects could not be due to rater bias. Nonshared environmental effects accounted for the remaining covariance over time. Genetic and shared environmental correlations across age of the reliable phenotype approached unity, indicating that the same genes and shared environment influence withdrawn behaviour throughout childhood.

Key words: genetics; twins; childhood; problem behaviour; longitudinal studies; heritability

Introduction

Children scoring high on withdrawn behavioural scales are characterised by shy, inhibited, introvert and withdrawn behaviour. Withdrawn behaviour (WB) correlates with symptoms of anxiety and depression (Verhulst et al., 1996), and WB in childhood has been shown to predict anxiety disorders and major depression in adolescence and adulthood (Goodwin et al., 2004). In a follow-up study spanning 14 years, Hofstra et al. (2000) found that parent reported WB was an important predictor for malfunctioning in adulthood. Withdrawn behaviour at the time of first measurement predicted both adult internalising and externalising problems 14 years later. Furthermore, inhibited 3-year-olds (children who are shy, fearful and easily upset) were more likely to meet diagnostic criteria for depression when they were 21 years old (Caspi et al., 1996). Children described as "shy" on multiple time points showed increased incidence of anxiety problems in adolescence (Prior et al., 2000). The evidence that childhood WB is a predictor for anxiety and depression later in life is further supported by laboratory studies of behavioural inhibition. Behavioural inhibition (characterised by shy, inhibited behaviour, and fear for novel situations) is present in about 10 to 15% of children (Kagan et al., 1988). Behaviourally inhibited children have higher rates of childhood anxiety disorders (Biederman et al., 2001; Rosenbaum et al., 1993) and are at increased risk of developing adolescent social phobia (Hayward et al., 1998).

The continuity of problem behaviours advocates research into the underlying mechanisms influencing stability of behavioural traits. An extensive line of research indicates that behavioural problems in childhood show considerable continuity. For example, in a study using parent reported problem behaviours it was found that 41% of the children classified as deviant at first assessment also showed behavioural problems in the clinical range 14 years later (Hofstra et al., 2000). Moreover, behavioural observations at age 3 appeared to be predictive of psychiatric disorders 18 years later (Caspi et al., 1996). Stability of problem behaviours is not confined to clinical groups but is also found in general population samples. In a large population sample of Dutch children, a correlation of .48 for problem behaviours across an 8-year period was found (Verhulst et al., 1996). In the last decades, a range of longitudinal studies have focused on childhood externalising problem behaviours in general (e.g. Bartels et al., 2004b; Fergusson, 1998; Haberstick et al., 2005; Van der Valk et al., 2003a) and more specific problem behaviours such as attention problems (e.g. Mannuzza et al., 2003; Rietveld et al., 2004), aggression (e.g. Alink et al., 2006; Campbell et al., 2006) or conduct disorder (e.g. Fergusson et al., 2005; Kim-Cohen et al., 2003). Likewise, substantial attention has been devoted to internalising behaviours in general (e.g. Bartels et al., 2004b; Haberstick et al., 2005; Van der Valk et al., 2003a) and more

narrowly defined problems such as anxiety disorder and depression (e.g. Fombonne et al., 2001; Tram & Cole, 2006). However, surprisingly little research has focused on withdrawn behavioural problems.

A powerful way of unravelling the genetic and environmental effects on individual differences in the development of behavioural problems is the study of genetically related individuals. Both cross-sectional and longitudinal studies using the classical twin design have been conducted to assess heritability estimates for broad band internalising and externalising problem behaviours (Bartels et al., 2004b; Van der Valk et al., 2003a) as well as for specific syndrome scales such as aggression (Haberstick et al., 2006; Van Beijsterveldt et al., 2003), obsessive compulsive disorder (Hudziak et al., 2004; Van Grootheest et al., 2007), juvenile bipolar disorder (Boomsma et al., 2006b), attention problems (Rietveld et al., 2004), and anxious/depression (Boomsma et al., 2005). However, no large scale longitudinal twin studies into WB have been reported.

Family studies into childhood WB have been scarce, but there are indications that familial factors play a role. Behavioural inhibition is more frequent in children whose parents have agoraphobia and panic disorder (Rosenbaum et al., 1988), and anxiety disorders are more frequent in the families of behaviourally inhibited children (Rosenbaum et al., 1991). Furthermore, a study in a large sample of 4-year-old twins reported a heritability of 76% for shyness/inhibition, as assessed with a 3-item questionnaire (Eley et al., 2003). A few twin studies examined the cross-sectional heritability of WB at various ages in childhood using the Child Behavior Checklist (CBCL; Achenbach, 1991; Achenbach, 1992). An early twin study in a relatively small sample of 2 to 3 year-old twins found no significant genetic effects on variance in WB (Schmitz et al., 1995). Contrary to these findings, Van den Oord et al. (1996) reported major genetic influences (74%) and no evidence for shared environmental influences on individual differences in WB in a sample of 1358 3-year-old twin pairs. Eight years later, Derks et al. (2004) analysed data on WB of more than 9000 3-year-old twin pairs, including the data used in Van den Oord's study and found moderate heritability (about 60% in boys; 45% in girls) and significant shared environmental effects. Two early twin studies examined the heritability of WB in middle childhood (sample sizes 181 and 203 pairs) and reported significant genetic effects (Edelbrock et al., 1995; Schmitz et al., 1995). On the other hand, a twin study from Taiwan including 279 12- to 16-year-old twin pairs (Kuo et al., 2004) found no significant genetic influences and major effects of shared and nonshared environment. One study compared WB data of biological and non-biological adopted siblings and found modest genetic influences at first assessment (age between 10-15 years) but no significant genetic effects three years later (Van der Valk et al., 1998).

These family studies are all based on parental ratings of WB. Using teacher report data of WB in 5-year-old twins, Polderman et al. (2006) found moderate genetic (49%) and nonshared environmental (51%) effects. Only two longitudinal studies (Schmitz et al., 1995; Van der Valk et al., 1998) have examined the genetic influences on the stability of childhood WB, and both failed to find significant genetic contributions to stability. However, in both studies the power to detect such effects was very low, due to limited sample size (Schmitz et al., 1995) or the design of the study (Van der Valk et al., 1998).

To summarise, the results of studies into the heritability of childhood WB have yielded varying results. Large scale studies into WB at later ages in childhood are lacking. Moreover, little is known about the genetic and environmental mechanisms underlying stability in WB. The aims of the current study are twofold. Firstly, this project, which is a follow-up of the twin sample studied by Derks et al. (2004), aims to examine the aetiology of variation in WB at various time points across childhood. Secondly, using the longitudinal nature of this study, we aim to assess the genetic and environmental factors underlying the stability of childhood WB.

Ratings of both maternal and paternal reported WB were incorporated in the analyses. Several studies into childhood behavioural problems have shown that different informants can provide different information about children's behaviour (Achenbach & Rescorla, 2000; Achenbach & Rescorla, 2001; Seiffge-Krenke & Kollmar, 1998; Van der Ende & Verhulst, 2005; Verhulst et al., 1996). Achenbach and Rescorla (2000; 2001) reported correlations between maternal and paternal ratings of WB of .69 for preschool children and of .57 for school-aged children. These correlations were based on data from a combined clinical and general population sample. In a general population only sample using the Dutch CBCL, the correlation between parental ratings of WB was found to be between .48 and .79 (Verhulst et al., 1996). The less-than-perfect correlation between parental ratings implies rater disagreement. Various studies have explored the sources of parental disagreement in ratings of problem behaviour (Bartels et al., 2003; Bartels et al., 2004a; Derks et al., 2004; Van der Valk et al., 2003b) using different structural equation models. Generally, it was found that parental agreement and disagreement was best explained by a psychometric model. This model, developed by Hewitt et al. (1992), assumes that parents not only assess the exact same behaviour of a child, but also rate an informant specific aspect of the child's behaviour. This unique perception of the child's behaviour can arise if the child behaves differently towards the different raters (e.g. the child is more withdrawn when it is with its mother than when it spends time with its father), or if the raters observe the child in different situations (e.g. the mother observes the child more often in the home environment, whilst the father often observes the child interacting with other children in the playground). Apart from these "real" differences

in behaviour, the unique perception of the child's behaviour may also be influenced by rater bias and unreliability. Rater bias may arise if parents hold on to different normative standards, have specific response styles, or tend to stereotype the child's behaviour. Unreliability may be an important source of rater disagreement if raters cannot give an accurate description of the behaviour under study. This may be relevant to our analyses, as some studies suggest that parents may be relatively insensitive to the more covert internalising problems of children (Ollendick & King, 1994; Seiffge-Krenke & Kollmar, 1998).

In the present study, stability of WB was assessed in longitudinal CBCL data from a large sample of 3, 7, 10 and 12 year-old twin pairs. The sample included roughly equal numbers of boys and girls. Genetic and environmental effects on stability in childhood WB were examined for both sexes. As both mother and father ratings of the twin's behaviour were incorporated in the analyses, we controlled for rater bias effects by distinguishing between variance that is shared between parents (i.e. perception of the child's behaviour common to both raters) and variance that is specific to one rater and might include variance due to rater bias. We compare the means and variance for WB in twins with estimates from a Dutch community sample (Van den Oord et al., 1995; Verhulst et al., 1996).

Methods

Participants

All participants were contacted via the Netherlands Twin register (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam (Bartels et al., 2007; Boomsma et al., 2002; Boomsma et al., 2006a). From 1986 onwards, the NTR has recruited families with multiples a few weeks or months after birth. Currently 40-50% of all multiple births are registered at the NTR. For the present study, data from twins born in 1986 – 2001 were included. Parents of the twins were asked to fill out a questionnaire assessing the twin's behaviour at age 3, 7, 10 and 12 years. The questionnaires were mailed within three months of the twin's 3rd, 7th, 10th and 12th birthdays. Two to three months after this mailing, reminders were sent to the non-responders. If finances permitted, persistent non-responders were contacted by phone. This procedure yielded a response rate between 61% and 73% (Bartels et al., 2007). Non-responders also include twin families who moved to an unknown address. From the original sample, 281 families were excluded because either one or both of the children had a disease or handicap that interfered with daily functioning. The total sample consisted of 14889 twin families. Ratings from both parents were available for 8479 families when the twins were 3 years old, 6414

at age 7, 4133 at age 10, and 2900 at age 12. Complete data from both parents at all time points were available for 1160 families. Maternal ratings were available for 14735 families, of which 13095 participated at age 3, 8855 at age 7, 5863 at age 10, and 3958 at age 12. Maternal data at all ages were available for 2797 families. Paternal ratings were available for 11499 families, of which 8794 families participated when the twins were 3 years old, 6522 at age 7, 4237 at age 10, 2974 at age 12. Complete father data on all four time points were available for 1290 families. This study is part of an ongoing project; the children born in later birth cohorts have not reached the age of 7, 10 or 12 years yet, which explains the decreasing numbers of participating families at the later ages.

To examine effects of sample attrition, we compared WB scores at age 3 of families who continued to participate at all other time points (when the twins were 7, 10, and 12 years of age), with families who only participated twice, once, or zero times at the subsequent time points. For the father ratings, there were no mean differences between these groups, neither for boys nor for girls. For the mother ratings, a significant effect of attrition was observed for both boys and girls. Mothers who continued to participate reported lower WB scores when their twins were 3 years old than mothers who did not participate at one or more of the later measurement occasions However, these effects were small (effect size r=.07 in both boys and girls).

Of all participating twin pairs, 2310 were monozygotic males (MZM), 2591 were dizygotic males (DZM), 2619 monozygotic females (MZF), 2339 dizygotic females (DZF), 2566 opposite sex twins with a male firstborn (DOSMF), and 2464 opposite sex twins with a female firstborn (DOSFM). For 1380 same sex twin pairs, zygosity was based on DNA polymorphisms (n=1039) or blood group (n=341; Van Dijk et al., 1996). For the remaining same sex twin pairs (n=8479), zygosity was determined by discriminant analysis, using longitudinally collected questionnaire items. This method has proven to be of sufficient reliability: Rietveld et al. (2000) reported that agreement between this method and zygosity determination by blood/DNA polymorphisms was 93%.

Measures

Mother and father ratings of WB problems were obtained from the withdrawn / depressed syndrome scale of the CBCL/2-3 at age 3. The CBCL/2-3 (Achenbach, 1992) has been translated and validated for the Dutch population (Koot et al., 1997). The withdrawn/depressed scale in the CBCL/2-3 consists of 10 items. The parents were asked to rate the behaviour of the child on a 3-point scale based on the occurrence of the behaviour in the past 2 months. They were asked to rate the behaviour as 0 if the problem item was not true; 1 if the item was somewhat or sometimes true; and 2 if it was very true or often true.

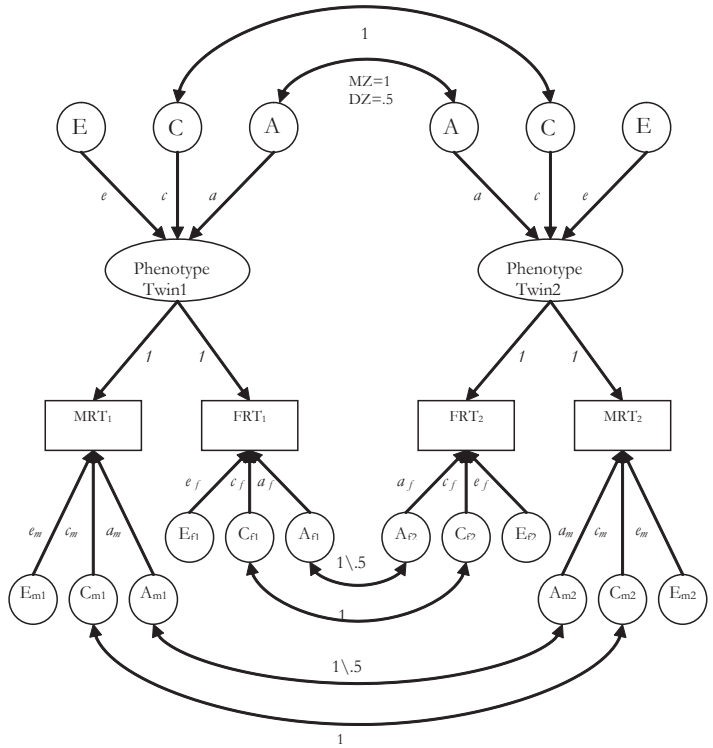

FIGURE 1. Psychometric model for multiple raters. A= Additive genetic effects; C = Shared environmental effects; E = Nonshared environmental effects.

At age 7, 10 and 12 WB was assessed using ratings of the WB syndrome scale of the CBCL/4-18 (Achenbach, 1991; Verhulst et al., 1996). The syndrome scale withdrawn encompasses 9 items, partially overlapping with the items in the CBCL/2-3. This time the parents were asked to rate the behaviour of the child in the preceding 6 months, on a 3-point scale identical to the scale used in the CBCL/2-3. For all ages, if more than two items on the WB scale were missing, the data were regarded incomplete and excluded from the analyses.

Data analyses

Descriptive statistics, correlations and cross- correlations for WB at age 3, 7, 10 and 12 years were estimated using the software package Mx (Neale et al., 2006). To assess stability of WB over time, phenotypic correlations between the time points were estimated for boys and girls separately. Furthermore, twin correlations at each age and cross-twin/cross-age correlations were estimated for each zygosity group separately. These correlations give a first impression of the contribution of genetic and environmental effects on the variance of WB at each age, and on the stability of

WB over time. Within-person inter-parent correlations were inspected to examine parental agreement on WB. Moreover, the cross-rater cross-twin correlations (e.g. the correlation between the mother rating of the oldest of the twins with the father rating of the youngest of the twins) were estimated to gain insight in the contribution of genes and environment on the phenotypic variance that both raters agree on.

Genetic modelling

Since monozygotic (MZ) twins are (nearly) genetically identical, while dizygotic (DZ) twins on average only share 50% of their segregating genes, genetic model fitting of twin data allows for separation of the observed phenotypic variance into its additive genetic (A) , shared environmental (C) and nonshared environmental (E) components. To incorporate the WB ratings from both parents into one model, a psychometric model was used (see Figure 1). The psychometric model enables a distinction between the variance that is shared by both raters and is independent of rater bias and unreliability (also called common phenotypic variance or reliable trait variance) and the variance that is rater specific (i.e. variance in the child's behaviour that is uniquely perceived by one of the parents, also called unique or rater specific phenotypic variance). In the psychometric twin model, both the common and the unique phenotypic variance are decomposed into genetic, shared environmental and nonshared environmental influences. Significant genetic effects on the rater specific variance indicate that these unique perceptions of the child's behaviour are "real", as error and unreliability do not cause systematic effects and cannot mimic genetic influences. Shared environmental effects on the unique phenotype may be confounded by rater bias, as possible influences of rater bias will act independently of the zygosity of the twins. Unique nonshared environmental influences may be confounded by measurement error or unreliability.

To examine the stability of WB throughout childhood, the psychometric model was extended to incorporate data on all four time points. To gain insight in what factors are important for the continuity of WB, a fully parameterised model (the Cholesky decomposition) was used. This model served as a reference to examine the significance of the different components. The deterioration of the model fit was evaluated after each component was dropped from the fully parameterised model using χ^2 tests. Genetic modelling was performed using Mx (Neale et al., 2006). In order to utilise all available data, including information of incomplete longitudinal data or data of which one of the parental ratings is missing, analyses were performed on the raw data.

TABLE 1. Sample sizes, means and standard deviations (SD) for mother and father ratings of withdrawn behaviour at age 3, 7, 10 and 12 for boys and girls separately.

	Mother ratings			Father ratings		
	N	Mean	SD	N	Mean	SD
			Age 3			
boys	12940	1.24	1.62	8686	1.16	1.26
girls	13078	1.09	1.43	8761	1.01	1.14
community boys	214	1.26	2.09			
community girls	199	1.02	1.54			
			Age 7			
boys	8698	1.66	1.70	6442	1.37	1.31
girls	8963	1.68	1.64	6578	1.37	1.29
community boys	579	1.61	1.75			
community girls	593	1.79	1.95			
			Age 10			
boys	5644	1.69	1.57	4116	1.35	1.27
girls	6043	1.62	1.53	4347	1.29	1.22
community boys	440	2.06	2.41			
community girls	456	2.10	2.32			
			Age 12			
boys	3836	1.53	1.46	2863	1.28	1.22
girls	4055	1.46	1.39	3068	1.16	1.18

Note: Data from the Dutch community sample are derived from Verhulst et al. (1996) and Van den Oord et al. (1995). At all ages, the majority (≥95%) of the children from the community sample were rated by their mothers.

Results

Table 1 shows the means and standard deviations for maternal and paternal rated WB, as assessed with the WB syndrome scale of the CBCL. Problem scores are shown for boys and girls from the twin sample, and from a community sample (Van den Oord et al., 1995; Verhulst et al., 1996). Mother ratings of WB are significantly higher than father ratings at all ages and in both boys and girls (all tests significant at

p<.001 level). Withdrawn behavioural problems are significantly higher in boys than in girls at age 3 (p<.001 for both raters), age 10 (mother rating p=.04; father rating p=.01) and age 12 years (mother rating p=.05; father rating p<.001), but not at age 7 years (mother rating p=.24; father rating p=.72). As the power to detect differences was very high in our study, these mean differences are only of practical importance at age 3. Furthermore, ratings of WB in our twin sample are similar to the scores in the community sample at age 3, 7, and 10 years. At age 12, the mean scores are higher in the community sample.

The phenotypic correlations are given in Table 2 for both the mother and the father ratings, separately for boys and girls. These correlations give an indication of the stability of WB over time. Phenotypic correlations are around .30 between age 3 and later ages, and increase to .44 - .65 between age 7, 10, and 12 years. This pattern is similar in both parental ratings and is observed in both boys and girls. Within-person cross-rater correlations (not shown in Table 2) were similar across zygosity and sex and were on average estimated as r=.53 at age 3, .56 at age 7, .57 at age 10 and .60 at age 12.

The twin correlations and cross-twin-cross-age correlations are presented in Table 3 for both the mother and the father ratings. Inspection of the MZ and DZ twin correlation (on the diagonal) at the four time points gives a first impression of what factors influence variance in WB. At all ages, MZ correlations are higher than DZ correlations in both sexes, indicating that genetic factors play a role. Twin correlations in opposite sex twins are similar to the correlations in DZ same sex twins, suggesting that there are no sex differences in genes or shared environment influencing WB. Apart from age 7, the MZ correlations are not twice as high as the DZ correlations, suggesting that shared environmental factors also play a role. Inspection of the MZ and DZ cross correlations (off-diagonal in Table 3) can provide insight in what fac-

TABLE 2. Phenotypic correlations for mother and father ratings of withdrawn behaviour in boys (above diagonal) and girls (below diagonal).

	Mother ratings				Father ratings			
Age	3	7	10	12	3	7	10	12
3	1	.33	.30	.29	1	.27	.23	.26
7	.32	1	.59	.51	.27	1	.54	.47
10	.27	.56	1	.65	.27	.55	1	.60
12	.27	.51	.59	1	.23	.44	.56	1

TABLE 3. Twin correlations and cross-correlations for mother and father ratings of withdrawn behavioural problems in six zygosity groups (MZM, DZM, DOSMF above diagonals; MZF, DZF, DOSFM below diagonals).

	Mother ratings				Father ratings			
Age	3	7	10	12	3	7	10	12
				MZF/MZM				
3	.72/.71[a]	.23	.21	.23	.73/.78[a]	.22	.20	.17
7	.29	.65/.60[a]	.42	.37	.29	.68/.64[a]	.38	.35
10	.22	.44	.63/.61[a]	.44	.26	.43	.63/.60[a]	.39
12	.27	.44	.47	.67/.63[a]	.23	.39	.36	.56/.63[a]
				DZF/DZM				
3	.47/.40[b]	.18	.20	.20	.50/.48[b]	.14	.14	.19
7	.20	.32/.27[b]	.23	.23	.21	.40/.29[b]	.18	.18
10	.23	.22	.36/.38[b]	.29	.24	.28	.44/.33[b]	.27
12	.21	.24	.28	.37.38[b]	.24	.23	.27	.41/.40[b]
				DOSFM/DOSMF				
3	.40/.45[c]	.16	.16	.16	.51/.50[c]	.16	.15	.18
7	.17	.37/.33[c]	.22	.18	.12	.39/.34[c]	.22	.18
10	.20	.25	.36/.35[c]	.22	.18	.28	.46/.32[c]	.26
12	.16	.23	.23	.34/.41[c]	.14	.19	.30	.31/.45[c]

Note: MZM = monozygotic males; DZM = dizygotic males; MZF = monozygotic females; DZF = dizygotic females; DOSMF = dizygotic opposite sex, male firstborn; DOSFM = dizygotic opposite sex, female firstborn. [a] First figure correlation MZF, second figure correlation MZM; [b] correlation DZF/DZM; [c] correlation DOSFM/DOSMF.

tors are important for the stability of WB over time. As compared to the DZ cross correlations, MZ cross correlations are slightly higher between age 3 and subsequent ages, and considerably higher between age 7 and later ages, indicating genetic effects on stability. However, MZ cross correlations are not twice as high, particularly between age 3 and later ages, suggesting that shared environmental effects on stability are also important.

TABLE 4. Cross-twin-cross-rater correlations within age (diagonal) and across age (off-diagonal) per zygosity group (MZM, DZM, DOSMF above diagonals; MZF, DZF, DOSFM below diagonals).

		Mother ratings			
	Age	3	7	10	12
			MZF/MZM		
Father ratings	3	41/.39[a]	.19	.18	.17
	7	.26	.40/.38[a]	.27	.27
	10	.22	.31	.36/.33[a]	.35
	12	.26	.30	.30	.39/.38[a]
			DZF/DZM		
	3	.27/.24[b]	.11	.12	.14
	7	.18	.18/.12[b]	.16	.14
	10	.20	.20	24/.22[b]	.19
	12	.15	.16	.21	.20/.26[b]
			DOSFM/DOSMF		
	3	.26/.26[c]	.12	.13	.13
	7	.10	.17/.14[c]	.12	.06
	10	.18	.15	.23/.18[c]	.13
	12	.08	.11	.14	.17/.24[c]

Note: MZM = monozygotic males; DZM = dizygotic males; MZF = monozygotic females; DZF = dizygotic females; DOSMF = dizygotic opposite sex, male firstborn; DOSFM = dizygotic opposite sex, female firstborn. [a] First figure correlation MZF, second figure correlation MZM; [b] correlation DZF/DZM; [c] correlation DOSFM/DOSMF.

Table 4 displays the cross-twin-cross-rater correlations within age (diagonal) and across age (off-diagonal). These correlations yield a first impression on the importance of genes and environment on the common phenotypic variance, and thus the reliable trait variance. At all ages, the MZ cross-rater correlations are larger than the DZ cross-rater correlations, indicating genetic effects on the common phenotype. Apart from the correlations at age 7, the DZ correlations are higher than would be expected based on genetic influences alone, therefore shared environmental influences also seem to influence the common phenotypic variance. For all ages the cross-twin-cross-rater correlations are lower than the cross-twin-within-rater correlations given in Table 3. These differences indicate parental disagreement, and reveal the part of the total variance that is due to a specific rater. Similar to the pattern of the within age twin correlations, the cross-twin-cross-rater correlations across age are higher in MZ than in DZ twins, indicating genetic effects. These correlations are less than

TABLE 5. Relative contributions of genetic (A), shared (C) and nonshared (E) environmental influences to the total (common + unique) variances (diagonal) and covariances (off-diagonal) of withdrawn behaviour for boys (above diagonal) and girls (below diagonal).

		Mother ratings				Father ratings			
	Age	3	7	10	12	3	7	10	12
A	3	.61/.62[a]	.83	.72	.75	.51/.61[a]	.87	.72	.81
	7	.71	.60/.64[a]	.80	.68	.75	.62/.66[a]	.78	.71
	10	.64	.74	.58/.59[a]	.68	.64	.63	.44/.52[a]	.48
	12	.55	.61	.67	.46/.53[a]	.60	.67	.43	.40/.50[a]
C	3	.15/.09[a]	.05	.10	.13	.23/.18[a]	.01	.10	.09
	7	.20	.05/.02[a]	.01	.07	.14	.05/.03[a]	.04	.04
	10	.21	.06	.05/.06[a]	.07	.21	.17	.19/.11[a]	.22
	12	.33	.16	.12	.19/.10[a]	.27	.07	.25	.19/.15[a]
E	3	.24/.28[a]	.12	.18	.12	.26/.21[a]	.12	.18	.10
	7	.09	.35/.34[a]	.19	.24	.11	.32/.31[a]	.18	.25
	10	.15	.20	.37/.36[a]	.25	.15	.20	.37/.37[a]	.30
	12	.12	.23	.21	.35/.38[a]	.13	.26	.32	.41/.35[a]

[a] First figure is the relative contribution for girls, second figure for boys

twice as high in MZ twins compared to DZ twins, especially in girls, indicating that shared environmental influences also play a role in the stability of the common phenotype. The cross-rater-cross-age correlations are similar to the within-rater-cross-age correlations between age 3 and later ages. This pattern indicates that most of the stability of WB is perceived by both raters. In later phases of childhood, the within-rater –cross-age correlations are larger than the cross-rater-cross-age correlations, indicating that both the common and the unique phenotype show considerable continuity over time.

The significance of all genetic and environmental components was tested by examining the deterioration of the model fit after each component was dropped from the fully parameterised model. All variance components were found to be significant,

TABLE 6. Relative contributions of genetic (A), shared (C) and nonshared (E) environmental influences to the variances (diagonal) and covariances (off-diagonal) of withdrawn behaviour for the common phenotype and the unique (rater specific) phenotype for boys (above diagonal) and girls (below diagonal).

		Mother ratings				Father ratings			
	Age	3	7	10	12	3	7	10	12
A_c	3	.28/.33[a]	.83	.72	.75	.30/.36[a]	.87	.72	.77
	7	.67	.35/.40[a]	.46	.68	.75	.39/.42[a]	.54	.68
	10	.64	.35	.17/.21[a]	.32	.64	.42	.20/.25[a]	.41
	12	.55	.59	.24	.22/.27[a]	.60	.67	.30	.25/.30[a]
C_c	3	.09/.04[a]	.01	.10	.07	.10/.05[a]	.01	.10	.07
	7	.09	.01/.00[a]	.00	.00	.10	.01/.00[a]	.01	.01
	10	.21	.05	.05/.03[a]	.03	.21	.05	.07/.03[a]	.04
	12	.24	.06	.10	.09/.02[a]	.27	.06	.13	.10/.02[a]
E_c	3	.07/.09[a]	.11	.18	.10	.08/.09[a]	.12	.18	.10
	7	.09	.12/.13[a]	.06	.24	.11	.13/.14[a]	.07	.25
	10	.15	.04	.04/.05[a]	.04	.15	.05	.04/.06[a]	.05
	12	.12	.23	.05	.10/.10[a]	.13	.26	.05	.11/.11[a]
A_u	3	.33/.29[a]	.00	.00	.00	.21/.25[a]	.00	.00	.04
	7	.04	.25/.24[a]	.34	.00	.00	.23/.24[a]	.24	.03
	10	.00	.39	.41/.38[a]	.36	.00	.21	.24/.27[a]	.07
	12	.00	.02	.43	.24/.26[a]	.00	.00	.13	.15/.20[a]
C_u	3	.06/.05[a]	.04	.00	.06	.12/.13[a]	.00	.00	.02
	7	.11	.04/.02[a]	.01	.07	.04	.04/.03[a]	.03	.03
	10	.00	.01	.00/.03[a]	.04	.00	.12	.12/.08[a]	.18
	12	.09	.10	.02	.10/.08[a]	.00	.01	.12	.09/.13[a]
E_u	3	.17/.19[a]	.01	.00	.02	.18/.12[a]	.00	.00	.00
	7	.00	.23/.21[a]	.13	.00	.00	.19/.17[a]	.11	.00
	10	.00	.16	.33/.31[a]	.21	.00	.15	.33/.31[a]	.25
	12	.00	.00	.16	.25/.28[a]	.00	.00	.27	.30/.24[a]

Note: A_c = Additive genetic influences on the common phenotype; C_c = Shared environmental influences on the common phenotype; E_c = Nonshared environmental influences on the common phenotype; A_u = Additive genetic influences on the unique phenotype; C_u = Shared environmental influences on the unique phenotype; E_u = Nonshared environmental influences on the unique phenotype. [a] First figure is the relative contribution for girls, second figure for boys.

for both the common phenotype and the unique phenotype (p<.001 for all components). Table 5 presents the relative contributions of genetic, shared and nonshared environmental influences to the total (common + unique) variances of WB at each age (diagonal) and to the stability of WB over time (off-diagonal). The heritability of both paternal and maternal rated behaviour is about 60% in both sexes at age 3 and age 7, and decreases slightly to 40-53% at the later ages. Shared environmental effects are of modest importance for the variance in both sexes, although the influence is slightly larger in girls. Nonshared environmental influences explain 21-41% of the variance at all ages. The relative importance of A, C and E to the total covariances between the four time points are shown on the off-diagonals of Table 5. Genetic effects are the driving force behind the stability of WB. Following the mother ratings of WB in boys, on average 74% of the stability ([83% + 72% +75% + 80% + 68% + 68%]/6=74%) is accounted for by genetic effects. Likewise, 65% of the maternal rated stability of WB in girls is explained by genetic effects. These contributions are similar for the paternal ratings. Shared environmental influences are important for the stability of WB in girls, and explain about 18% of the behavioural continuity in both mother and father ratings. In boys, these effects only explain about 8% of the covariance. The remaining part of the covariance is explained by nonshared environmental effects.

In Table 6, a distinction is made between the contributions of A, C and E to the common phenotype (A_c, C_c, and E_c), and to the phenotype unique to either the mother or the father ratings (A_u, C_u, E_u). At age 3 and age 7, the common and the unique phenotypic variance contribute equally to the total variance. At later ages in childhood, substantial extra information is added by the specific raters, especially by the mothers. A large proportion of the rater specific variance is due to genetic influences, indicating that this rater specific information of the child's behaviour is real. About half of the shared environmental influences on WB is rater specific. These effects may be real rater specific shared environmental effects, but may also be due to rater bias. The off-diagonals of Table 6 show the influences of common and rater specific genetic and environmental influences on the covariances. Common genetic influences are most important for explaining continuity of WB, and account for about 55% of the total covariance in girls and 65% in boys. Table 6 also shows that most of the shared environmental influences to stability are common to both raters. This indicates that these effects are "real" and not due to rater bias. On the whole, the possible effects of rater bias are small in this study, as rater specific shared environmental effects on average only explain 4% (boys) to 5% (girls) of the stability.

Lastly, the correlations between the genetic influences over time and the correlations between shared and nonshared environmental effects across development are displayed in Table 7. The genetic correlation of the common phenotype remains high

TABLE 7. Genetic, shared and nonshared environmental correlations across time for boys (above diagonal) and girls (below diagonal).

Age	3	7	10	12	3	7	10	12	3	7	10	12
	A_c				A_{um}				A_{uf}			
3	-	.86	1.00	.84	-	.00	.00	.00	-	.00	.00	.05
7	.76	-	.86	.96	.06	-	.62	.00	.00	-	.48	.08
10	1.00	.76	-	.84	.00	.64	-	.71	.00	.45	-	.18
12	.70	.99	.70	-	.00	.04	.75	-	.00	.00	.34	-
	C_c				C_{um}				C_{uf}			
3	-	1.00	1.00	.79	-	.56	.00	.31	-	.00	.00	.07
7	1.00	-	1.00	.79	.85	-	.22	.94	.18	-	.33	.29
10	1.00	1.00	-	.79	.00	.50	-	.47	.00	.83	-	1.00
12	.85	.85	.85	-	.38	.81	.89	-	.00	.05	.57	-
	E_c				E_{um}				E_{uf}			
3	-	.39	1.00	.37	-	.01	.00	.02	-	.00	.00	.00
7	.35	-	.39	1.00	.00	-	.28	.00	.00	-	.25	.00
10	1.00	.35	-	.37	.00	.30	-	.45	.00	.32	-	.51
12	.42	1.00	.42	-	.00	.00	.30	-	.00	.00	.45	-

Note: A_c = Additive genetic influences on the common phenotype; C_c = Shared environmental influences on the common phenotype; E_c = Nonshared environmental influences on the common phenotype; A_u = Additive genetic influences on the unique phenotype as rated by the mother (A_{um}) or the father (A_{uf}); C_u = Shared environmental influences on the unique phenotype rated by the mother/father (C_{um}/C_{uf}); E_u = Nonshared environmental influences on the unique phenotype rated by the mother/father (E_{um}/E_{uf}).

across time, indicating that roughly the same genes influence the stability of the reliable WB phenotype between age 3 and age 12 years. Likewise, the shared environmental effects on the stability of the common phenotype remain largely the same over time. The nonshared environmental correlations and the rater specific genetic and shared environmental correlations over time are lower. These effects are thus more variable over time.

Discussion

We studied the aetiology of WB in a large sample of 3-, 7-, 10-, and 12-year-old twins, and explored the genetic and environmental influences on stability of WB across childhood. Both maternal and paternal ratings of their children's behaviour were analysed in order to identify the part of the phenotype that both raters agree on, and correct for possible rater bias.

Individual differences in WB were largely influenced by genetic effects at all ages, in both boys (heritability estimates 50-66%) and girls (heritability estimates 40-61%). Shared environmental influences explained a small to modest proportion (2 – 23%) of the variance at all ages in both sexes, but were slightly more pronounced in girls. Nonshared environmental influences were of moderate importance to the variance at all ages in both boys (21-38%) and girls (24-41%). This study is the first large scale study examining genetic and environmental influences on WB at multiple time points in childhood which is adequately powered to test for shared environmental effects. Results from previous family studies into childhood WB provided varying results. Two earlier twin studies in 3-year-olds from the NTR (Derks et al., 2004; Van den Oord et al., 1996), found significant genetic effects, varying in magnitude from 45 to 74%. The most recent study with the largest sample size (Derks et al., 2004) also found significant shared environmental effects that were more pronounced in girls than in boys. Two studies in middle to late childhood reported heritabilities of 40% (Schmitz et al., 1995) and 53% (Edelbrock et al., 1995). Two other family studies in middle to late childhood found no or little genetic effects (Kuo et al., 2004; Schmitz et al., 1995; Van der Valk et al., 1998). In the Taiwanese twin study (Kuo et al., 2004) the 95% confidence interval for additive genetic effects, however, was between 0 and 55%, indicating that the proportion of the variance explained by additive genetic influences may have been as large as 55%. On the other hand, the different results in the Taiwanese study compared to ours may also be due to cultural differences between the populations. Previous studies have suggested cultural effects on WB (Crijnen et al., 1999; Murad et al., 2003).

The longitudinal nature of the current study allowed for examination of the stability of WB throughout childhood. Withdrawn behaviour showed considerable continuity over time, with stability coefficients ranging from .30 between age 3 and 12 years to .65 for the shorter time intervals. These phenotypic correlations were similar to the correlations reported in other studies of childhood WB. In a small longitudinal twin sample, Schmitz et al. (1995) found a correlation of .33 between CBCL/2-3 scores and CBCL/4-18 scores of WB. In a large general population sample from the Netherlands (Verhulst et al., 1996), an 8-year stability coefficient of .36 was reported.

Smaller time intervals gave higher stability coefficients (.47 for a 6-year interval; .46 for a 4 year interval and .60 for a 2-year interval).

We studied the genetic and environmental influences underlying stability in childhood WB. Stability in WB problems was largely accounted for by genetic effects, in boys (about 74% across time) and girls (about 65%). In girls, the shared environment was of moderate importance for continuity of WB, these influences explained on average 18% of the stability over time. In boys, these effects were less important, explaining about 8% of the stability. Interestingly, most of the shared environmental influences on stability were common to both raters, indicating that these effects are not due to rater bias. Nonshared environmental effects explained 17 to 20% of the stability over time in both sexes. Around two third of these effects were common to both raters.

In a longitudinal study into childhood internalising behaviour in this same sample, Bartels et al. (2004b) found that stability in internalising problems was accounted for by both genetic and shared environmental effects, and these effects were roughly of the same importance for stability (43 vs. 47%). We found that genetic effects largely explained stability in WB (65% in girls; 74% in boys), whilst shared environmental influences were only of modest importance (8-20%). The broad band internalising problem behaviour scale of the CBCL/2-3 includes the Withdrawn and Anxious/Depressed syndrome scales. The internalising problems scale in the CBCL/4-18 consists of the subscales Anxious/Depressed; Somatic complaints and Withdrawn. Bartels et al. (2004b) found decreasing genetic effects and increasing shared environmental effects on the variance in internalising behaviour over time. The same pattern was found in a longitudinal study into Anxious/Depressed behaviour in childhood (Boomsma et al., 2005). Our study suggest that, unlike the other syndrome scales that make up the broad band internalising scale, for WB shared environmental effects do not become increasingly important in later childhood.

The correlation between the genetic influences on the common phenotype over the course of development approaches unity. As the common phenotype represents the behaviour that both raters agree upon, and can thus be considered as a reliable phenotype, the high genetic correlations suggest that the same genes influence the continuity of WB over time. Similarly, the shared environmental correlation of the common phenotype is close to 1.0 over time, indicating that a stable persistent shared environmental influence is of importance for behavioural stability. Nonshared environmental correlations on the other hand vary over time in both boys and girls, suggesting that these effects are less persistent. The family environment could be an important factor for stability. Some studies suggest that parental behaviour may moderate the stability of behavioural inhibition and shyness in young children. Inappropriate affectionate parenting (Park et al., 1997) or maternal over-control

(Rubin et al., 2002) could increase stability of these behaviours. The family environment can show up in the shared environmental component (i.e. socioeconomic status of the family, parental rearing practices) or in the nonshared environmental component (i.e. child specific parenting). Nonshared environmental influences, such as traumatic experiences, the consequences of an accident or illness could also account for stability in WB.

The extent to which a child displays WB might be influenced by the composition of the family in which the child is raised. Our study focused on variation in WB in twins. It may be that twins show less WB compared to singletons, because they are raised with a sibling of the exact same age. On the other hand, if twins mainly interact with each other in childhood, twins may be more inhibited than non-twin siblings or singletons in interaction with others than their co-twin. Comparing the mean scores of our twin sample with the scores in a Dutch community sample showed that withdrawn scores are similar at age 3, 7 and 10 years. At age 12, however, mean problem scores were higher in the community sample than in the twin sample. In a large twin-singleton comparison study (Pulkkinen et al., 2003), 12-year-old twins were reported to be more socially adaptable than non-twins, but no twin-singleton differences were found for social anxiety. A twin-singleton comparison of both maternal CBCL withdrawn ratings and laboratory assessment of inhibition in 5-year-olds yielded inconsistent results (DiLalla & Caraway, 2004). According to laboratory ratings, twins were more inhibited than non-twins, whilst maternal ratings showed the opposite. These studies yield no explanation for the low mean withdrawn scores observed in our twin sample at age 12. To explore possible twin-singleton differences, future studies are needed.

The current study highlights the importance of genetic effects on stability in WB. Unlike the other internalising behaviours, shared environmental influences do not become increasingly important throughout childhood. Results from longitudinal (Bongers et al., 2003) and cross-sectional studies (Achenbach, 1991; Verhulst et al., 1996) indicate an increase in WB from childhood to adolescence. Future longitudinal research should extend the current study and investigate stability of WB into adolescence. Since childhood WB has been shown to be a predictor for anxiety disorders and depression later in life (Goodwin et al., 2004), insight into the developmental mechanisms underlying stability of WB into adolescence would be highly desirable. This is particularly true given the differences between the Anxious/Depressed and WB scales of the broad band internalising scale. It appears from our studies that WB has a different genetic architecture and longitudinal course than Anxious/Depressed. While Anxious/Depressed has received a great deal of research attention, there has been relatively little investigation into the long term sequelae of WB. Our research provides evidence for the need for further work on this phenotype.

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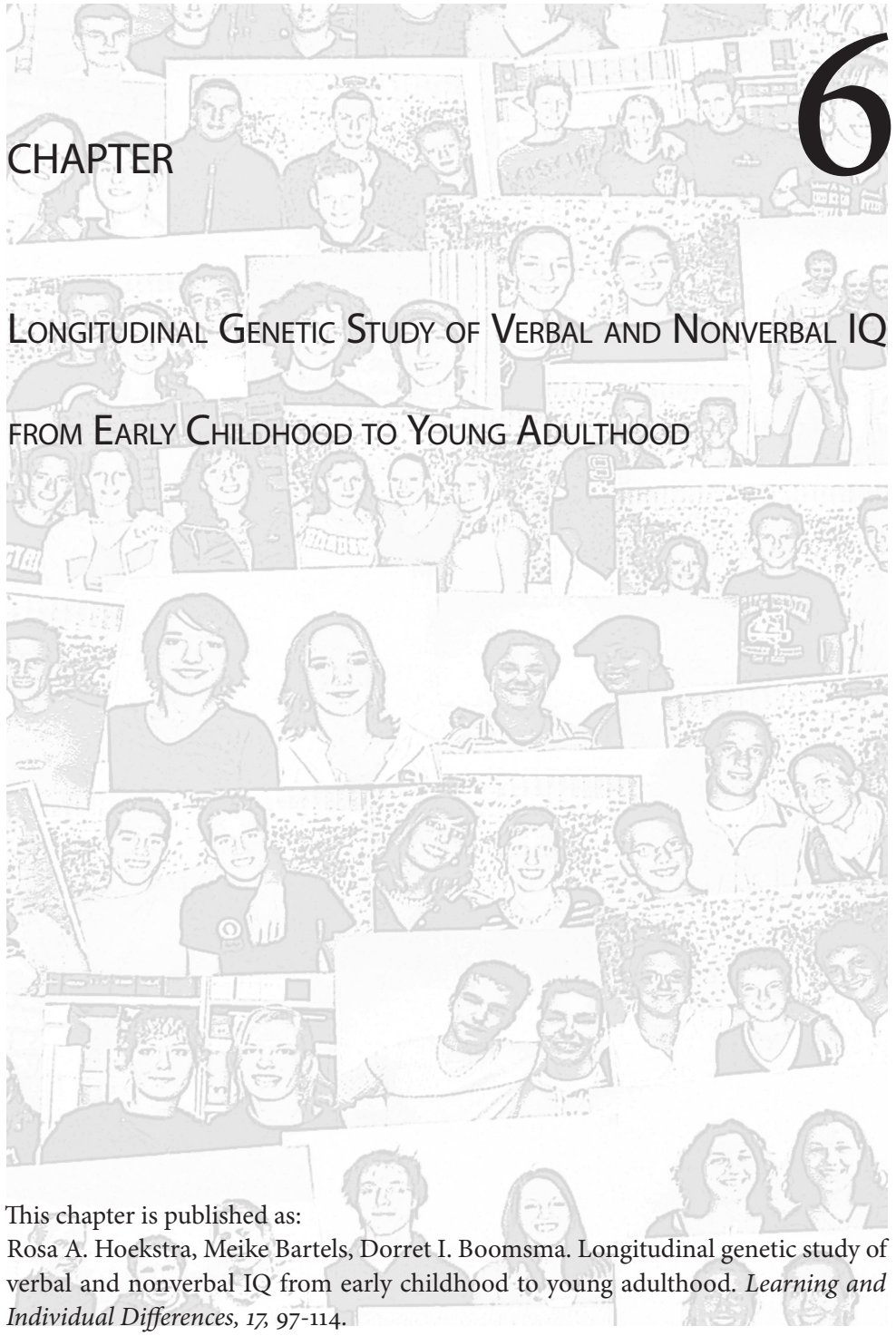

CHAPTER 6

LONGITUDINAL GENETIC STUDY OF VERBAL AND NONVERBAL IQ FROM EARLY CHILDHOOD TO YOUNG ADULTHOOD

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Abstract

In a longitudinal genetic study we explored which factors underlie stability in verbal and nonverbal abilities, and the extent to which the association between these abilities becomes stronger as children grow older. Measures of verbal and nonverbal IQ were collected in Dutch twin pairs at ages 5, 7, 10, 12 and 18 years. The stability of both verbal and nonverbal abilities was high, with correlations over time varying from .47 for the 13-year time interval up to .80 for shorter time intervals. Structural equation modelling showed increasing heritability with age, from 48% (verbal) and 64% (nonverbal) at age 5 to 84% and 74% at age 18. Genetic influences seemed to be the driving force behind stability. Stability in nonverbal ability was entirely explained by genes. Continuity in verbal abilities was explained by genetic and shared environmental effects. The overlap between verbal and nonverbal abilities was fully accounted for by genes influencing both abilities. The genetic correlation between verbal and nonverbal IQ increased from .62 in early childhood to .73 in young adulthood.

Introduction

General cognitive ability, or intelligence, is one of the best studied areas in behaviour genetics (see for reviews Bouchard, Jr. & McGue, 2003; Deary et al., 2006; Plomin & Spinath, 2004). Twin family and adoption studies have examined genetic and environmental influences on cognition at several time points across the life span. It is well established that genetic factors increase in importance over the life time, whilst shared environmental influences diminish. The heritability of general cognitive ability in infancy is estimated at about 20% (Bishop et al., 2003; Fulker et al., 1988; Petrill et al., 2004a; Spinath et al., 2003; Wilson, 1983), increases to about 40% in middle childhood (e.g. Bartels et al., 2002) and may be as high as 80% in adulthood (e.g. Posthuma et al., 2001; Rijsdijk et al., 2002). In parallel, shared environmental influences explain about half of the variance in intelligence in young children (Bartels et al., 2002; Spinath et al., 2003), decrease in importance at later ages in childhood (Bartels et al., 2002), and become non-significant by adolescence (e.g. Posthuma et al., 2001; Rijsdijk et al., 2002; Scarr & Weinberg, 1983).

Stability of general cognitive ability

Longitudinal studies show that general cognitive ability is a highly stable trait. A 68-year follow-up of almost 500 people showed a stability coefficient of .66 between IQ scores on a test taken at age 11 years and 79 years (Deary et al., 2004). Cognitive ability in childhood (age 5 – 12) shows similar stability (Bartels et al., 2002), but tests conducted at very young ages may be less predictive of cognitive abilities in later life (Bishop et al., 2003; Petrill et al., 2004a), although these results have been challenged. The lack of prediction may stem from the fact that traditional measures of infant IQ, such as the Bayley Mental Development Index, are poor predictors of later IQ scores (Boomsma, 1993). Measures of infant cognitive function, such as habituation and novelty preference seem more predictive of later IQ (Bornstein & Sigman, 1986). DiLalla et al. (1990) measured novelty preference in twins 7, 8 and 9 months. Mid-twin scores were regressed on mid-parent WAIS-IQ and showed significant heritability at 9 months. Spinath et al. (2003) assessed verbal and nonverbal abilities in a longitudinal twin study at age 2, 3, and 4 years and reported 2-year stability coefficients ranging from .36 to .49. The authors of this study also performed a principal component analysis to derive a general intelligence (or "g") factor. The 2-year stability of g was found to be .60, suggesting that general cognitive ability can also be measured reliably in early life.

Developmental mechanisms underlying stability of general cognitive ability

Longitudinal twin and family studies enable disentangling genetic and environmental influences on the stability of cognitive abilities over time. The genetic and environmental influences may exert their effect on stability following different developmental mechanisms. Firstly, the same genetic or environmental influences may affect IQ throughout development, although their relative importance can change over time. This structure suggests an underlying factor (genetic or environmental) that influences cognitive ability at each time point and accounts for stability of intelligence over time. This type of developmental structure in genetic modelling is modelled as a common factor (Martin & Eaves, 1977; see Bartels et al., 2002 for a recent application). Secondly, genetic or environmental influences may exert their effects by carrying over part of prior experiences to subsequent ages, together with new influences, or innovations, at each occasion. In this pattern, the influences on intelligence at successive ages are causally linked, so that each new event builds upon earlier experiences. Stability of intelligence over time is explained by the part of earlier influences that is transmitted to subsequent ages. Innovations, e.g. new genes that are expressed, can enter at each age. This developmental pattern is referred to as a transmission structure, or simplex model (Boomsma & Molenaar, 1987; see Bartels et al., 2002 for a recent application). This model is suggested when the phenotypic correlations decrease with longer time intervals (Jöreskog, 1970). Lastly, genetic and environmental influences may be specific to a certain time point only and not exert effects on the continuity of cognitive ability. These effects are referred to as age specific affects.

Different research groups have conducted twin and adoption studies of cognitive development. We focus here on the most recent findings of twin and adoption studies spanning a long time of development. The Louisville Twin Study (LTS), initiated nearly 50 years ago, includes almost 500 twin pairs and their siblings who have participated in a longitudinal study of cognitive development from age 3 months through 15 years (Wilson, 1983). Data from this study suggest that the continuity of cognitive ability is largely explained by genetic and shared environmental effects (Eaves et al., 1986), whilst nonshared environmental effects are occasion specific. The Colorado Adoption Project (CAP) has collected data on adopted children and their adoptive and biological parents and on non-adoptive (control) families. Reports up to now include data on cognitive development spanning age 1 to 16 years (Petrill et al., 2004a). In this longitudinal data set, stability in general cognitive ability was mainly accounted for by genetic effects. The genetic stability was accounted for by a common facture structure. Shared environmental effects were not significant, whereas nonshared environmental influences were mainly age specific. Bishop et al. (2003) studied cognitive development from age 1 to 12 years in a combined sample of the

above mentioned CAP study and a longitudinal twin sample. They reported a transmission structure for genetic influences in early ages of development, changing into a common factor structure in later childhood. These genetic effects accounted for most of the stability in cognitive ability. Furthermore, a small shared environmental effect was found, that contributed to stability mainly from infancy through early childhood via a common factor pattern. Nonshared environmental influences were mainly age specific but also accounted for some stability in middle childhood. Bartels et al. (2002) studied cognitive development from age 5 to 12 years in a longitudinal twin sample from the Netherlands, overlapping with the sample used in the current paper. They reported a common factor structure for genetic influences, accounting for stability in total IQ at all ages. Shared environmental effects influenced stability as well as change via a common factor structure and age specific influences, whilst nonshared environmental influences were only age specific. The overall picture that can be drawn from these studies is that stability in cognitive ability is mainly accounted for by genetic effects. The nonshared environment is only of importance for effects specific to each time point and does not contribute to stability of cognitive abilities.

Specific cognitive abilities

Although genetic and environmental effects on the development of general intelligence are well documented, less is known about the development of specific cognitive abilities. A hierarchical organisation of cognitive abilities is now widely recognised. A general cognitive factor accounts for about 50% of the variance in a broad variety of cognitive tests (Carroll, 1993; Deary, 2001). When this variance is taken into account, the remaining variance tends to cluster together into separable group factors of intelligence. Often, cognitive abilities are separated into verbal and nonverbal abilities (e.g. Wechsler intelligence scales verbal IQ (VIQ) and performance IQ (PIQ); Wechsler, 1997); or into more specific factors encompassing verbal comprehension, perceptual organisation, working memory, and processing speed (Wechsler, 1997).

In adults, verbal abilities appear to be somewhat more heritable than nonverbal abilities. In two twin studies in young adults (Rijsdijk et al., 2002) and a sample of young and middle aged adults (Posthuma et al., 2001) heritability estimates for VIQ and PIQ were 84% and 85% for VIQ, and 68% - 69% for PIQ. In the latter twin sample (Posthuma et al., 2003), verbal comprehension was found to be somewhat more heritable (84%) than perceptual organisation (68%), working memory (65%) and processing speed (63%). The Hawaii Family Study of Cognition, including data from 1816 families from American/European or Japanese ancestry, is one of the largest samples in which familial transmission of special cognitive abilities has been studied (DeFries et al., 1979). The midparent-offspring resemblance in both samples was

higher for verbal (.48 - .54) and spatial (.60 - .42) abilities than for perceptual speed (.41 - .34) and memory (.31 - .18) factors (cited from Alarcón et al., 2003). Two twins-reared-apart studies (McGue & Bouchard, Jr., 1989; Pedersen et al., 1992) reported heritabilities of 57 - 58%% (verbal abilities), 71 - .46% (spatial abilities), 53 -.58% (perceptual speed) and 42 - .38% (memory). Similar to general cognitive abilities, the heritability of specific cognitive abilities seems to increase with age. Whilst the heritability of verbal and nonverbal abilities is about 25% in infants (Price et al., 2000) the heritability increases to about 40% in middle childhood (Rietveld et al., 2003). Results from the LTS sample (Wilson, 1986) showed increasing monozygotic twin correlations for VIQ scores from age 5 to 15 years, whilst the dizygotic twin correlations remained stable. This pattern suggests increasing heritability over time. The heritability also increased with age for PIQ, but the twin correlations were somewhat lower than for VIQ, suggesting a larger influence of the nonshared environment. The CAP project reported a heritability of verbal, spatial, memory and perceptual speed abilities varying from 6 to 31% in 4-year-olds (Rice et al., 1989). These estimates increased to 19 – 35% in 7-year-olds (Alarcón et al., 2003), to 26 – 53% in 12-year-olds (Alarcón et al., 1998; Alarcón et al., 2003), and to 32-64% when the offspring was 16 years old (Alarcón et al., 1999). Longitudinal model fitting of the CAP data in 3 to 9-year-old children (Cardon, 1994) suggested that genetic effects are of main importance for the stability in specific cognitive abilities, exerting their effects via a transmission structure.

With multivariate genetic analyses, the extent to which genetic or environmental influences account for overlap between specific cognitive abilities can be examined. Such studies have found that genetic correlations (r_g, the extent to which genetic effects on one trait correlate with genetic effects on another trait) among specific cognitive abilities are substantial in adulthood (r_g ranging from .35 to .87, depending on the tests used; Posthuma et al., 2001; Posthuma et al., 2003; Rijsdijk et al., 2002) and in middle to late childhood (r_g varying from .27 to .79; Alarcón et al., 1998; 1999; Casto et al., 1995). In contrast, a study in infancy found a genetic correlation between verbal and nonverbal abilities of around .30 (Price et al., 2000). These findings suggest that genetic effects on specific cognitive abilities are largely independent in infancy, and become increasingly more correlated in later stages of cognitive development (Petrill, 1997; Petrill et al., 2001; Plomin & Spinath, 2002; Price et al., 2000). However, these results are based on cross-sectional comparisons.

Aims of the present study

The current paper reports on a longitudinal twin study of cognitive development spanning early childhood to young adulthood. Factor analyses of the data assessed at the first measurement occasion (when the twins were 5 years old), revealed a verbal and a nonverbal factor (Rietveld et al., 2000). A longitudinal analysis of the first three assessments when the twins were respectively 5, 7, and 10 years old (Rietveld et al., 2003) showed that stability in verbal and nonverbal ability was mainly due to genetic effects. The nonshared environment contributed to age specific variance only. The genetic correlation between verbal and nonverbal factors increased slightly over the years, but was still low at age 10 (r=.25 at age 5, to r=.30 at age 10), and of similar magnitude as the genetic correlation reported by Price et al. (2000). The current report is a follow-up of this study and includes assessment of verbal and nonverbal abilities at age 12 and 18 years in the same sample. This study aims to 1) examine genetic and environmental influences on verbal and nonverbal abilities at 5 time points spanning development from age 5 to 18 years; 2) Explore the developmental structure underlying stability in verbal and nonverbal abilities; 3) Examine to which extent genetic effects influence the overlap between verbal and nonverbal abilities and to test if there is an increase in this correlation over development, as suggested by previous cross-sectional studies.

Methods

Participants

This project is part of an ongoing longitudinal study into the development of intelligence and problem behaviour. The study was initiated in 1992 with the recruitment of 209 5-year-old twin pairs from the Netherlands Twin register (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam (Boomsma et al., 1992; Boomsma et al., 2002). The twin families were selected on the basis of age, zygosity of the twins, and their place of residence. Mean age at the first measurement occasion was 5.3 years (sd=0.2). At the second measurement occasion (mean age 6.8 years, sd=0.2) 192 pairs of the initial sample completed the test protocol. Around the tenth birthday of the twins (mean age 10.0 years, sd=0.1) 197 twin pairs participated in the third data collection. The fourth assessment (mean age 12.0 years, sd=0.1) was completed by 192 twin pairs. Six years later, 122 twin pairs of the initial sample participated in the fifth measurement occasion (mean age 18.1 years, sd=0.2). To increase the sample size on the fifth assessment, 64 additional twin pairs (mean age 18.3 years, sd=0.1) were recruited via the NTR. Complete data on all 5 measurement occasions were available for 115 twin pairs. No significant differences in verbal and nonverbal IQ at age 5 were found for subjects who did not wish to participate in one of the assessments at age 7, 10 or 12 years ($F(3, 203)=.663$, $p=.576$ for verbal IQ; $F(3, 205)=1.660$, $p=.177$ for nonverbal IQ). However, subjects who continued to participate at age 18 had higher mean verbal ($F(1, 205)=7.834$, $p=.006$, $d=.40$)

and nonverbal (F(1, 207)=4.471, p =.036, d=.30) IQ scores at age 5 as compared to subjects who did no longer take part when they were 18 years old. The vast majority of the twins still lived with one or both of their parents at age 18 years.

Of all twin pairs from the longitudinal sample, 42 were monozygotic males (MZM), 44 were dizygotic males (DZM), 47 monozygotic females (MZF), 37 dizygotic females (DZF), and 39 dizygotic twin pairs of opposite sex (DOS). For the same sex twin pairs, zygosity was based on blood group polymorphisms (63 pairs) or DNA analyses (100 pairs). For the remaining twins, zygosity was determined by physical resemblance assessed by an experienced test administrator (4 pairs) or by discriminant analyses of longitudinally collected questionnaire items (3 pairs). Of all newly recruited families that only participated at age 18, there were 13 MZM twin pairs, 12 DZM pairs, 16 MZF pairs, 9 DZF pairs and 14 DOS twin pairs. Zygosity determination in the same sex twins of this group was based on DNA analysis (37 pairs), blood group polymorphisms (7 pairs) or questionnaire items (7 pairs).

Procedures and intelligence tests

At ages 5 and 7, the twins participated in a study on the development of cognitive abilities and brain activity (Boomsma & Van Baal, 1998; Van Baal et al., 2001). At both measurement occasions, the twins visited the university laboratory. While one of the twins participated in the electrophysiological experiment, the co-twin completed the intelligence test. At ages 10 and 12, the intelligence tests were conducted either at the twins' home or at the university, depending on the preference of the twin family. Most of the families preferred testing at home (around 70% at both ages). There were no significant differences in intelligence between the twins tested at home and the twins tested at the university (Bartels et al., 2002). At age 18 the children visited the university to complete the intelligence test as part of an extensive test protocol, including assessment of physical development and neuropsychological tasks. At all ages, the intelligence test was administered by experienced test administrators. At ages 5, 7, and 10, the test took approximately 1 hour to complete, and, at ages 12 and 18 the test took 1.5 hours to complete. At the end of each test protocol the twins received a present.

At age 5, 7, and 10 years, the children completed the Revised Amsterdamse Kinder Intelligentie Test (RAKIT; Bleichrodt et al., 1984). The RAKIT is a Dutch psychometric intelligence test for children, with subtests covering a broad spectrum of intellectual abilities. The test is designed for children in the age of 4 to 11 years. The short version of the RAKIT was used, which has six subtests with age-appropriate items, measuring verbal and nonverbal abilities. Both the verbal and nonverbal IQ scores were based on the sum of three subtests scores, which were transformed into standardised scores. The standardisation was based on a population sample of Dutch 6- to

11-year-old children; the norms for standardisation were the same for boys and girls. For further details on this intelligence test, see Rietveld et al. (2003).

At age 12 the Dutch version of the Wechsler Intelligence Scale for Children–Revised (WISC-R; Van Haassen et al., 2006) was used. The complete test was conducted, encompassing 6 verbal and 6 nonverbal subtests. The WISC-R is an internationally used psychometric intelligence test and can be used from age 6 to age 16 years. Standardised verbal and nonverbal IQ scores were based on results of same-aged children in the Netherlands. The transformation from raw scores into standardised scores was based on the same norms for boys and girls.

At age 18 the Dutch version of the Wechsler Adult Intelligence Scale-third edition (WAIS-III; Wechsler, 1997) was administered. The twins completed 11 subtests, including 6 verbal and 5 nonverbal tests. The subtests were standardised for the appropriate age group, based on a population sample of same-aged subjects in the Netherlands. Standardisation norms were the same across the sexes. Verbal and nonverbal ability scores were calculated as the mean subtest score on the 6 verbal, respectively the 5 nonverbal subtests. The concurrent validity of the RAKIT and the WISC-R is .86 (Pijl et al., 1984). The correlations between VIQ and PIQ scores measured with the WISC-R and the WAIS-R are high (.89 for VIQ, .76 for PIQ; Wechsler, 1981).

Statistical analyses

All analyses were carried out with structural equation modelling as implemented in the software package Mx (Neale et al., 2006). To assess stability of verbal and nonverbal IQ over time, and the association between verbal and nonverbal abilities at all ages, phenotypic correlations were estimated in a saturated model. All data, regardless of the pattern of missingness, were analysed using the raw data option in Mx. By analysing all data, any bias that may have been introduced by non-random drop out is corrected for (Little & Rubin, 2002). Twin correlations at each age and cross-twin/cross-age correlations were also estimated in the saturated model. These correlations give a first impression of the contribution of genetic and environmental effects on the variance of verbal and nonverbal abilities at each age, and on the aetiology of stability of these traits over time. The cross-twin/cross-trait correlation (i.e. the correlation between verbal IQ in the one twin with nonverbal IQ in the co-twin) was also estimated at each age. These correlations give a first indication of the relative importance of genes and environment on the overlap between verbal and nonverbal abilities. Furthermore, it was tested whether the correlation patterns for verbal and nonverbal abilities were different across the sexes.

Genetic modelling

Monozygotic (MZ) twins are genetically identical at the DNA sequence level (but may show differences in gene expression due to e.g. differences in DNA methylation patterns; Jirtle & Skinner, 2007). Dizygotic (DZ) twins share on average 50% of their segregating genes. This experiment of nature allows statistical modelling of twin data with the goal to attribute the observed variance into genetic and environmental contributions. Additive genetic variance (A) is the variance that results from the additive effects of alleles at each contributing genetic locus. Dominant genetic variance (D) is the variance that results from within locus interaction of the alleles at all contributing loci. Shared environmental variance (C) is the variance resulting from environmental effects common to both members of a twin pair. Nonshared environmental variance (E) is the variance caused by environmental influences that are not shared by members of a twin pair. Estimates of the unique environmental influences also include measurement error. To take this source of variance into account, E is always specified in the model. Using twin data, the influence of C and D cannot be estimated simultaneously. However, comparing the twin correlations of MZ and DZ twins can give a first indication of what influences are important. If MZ and DZ twin correlations are similar, shared environmental influences are likely to be important. Conversely, a DZ twin correlation that is less than half the MZ twin correlation indicates dominance effects. Likewise, if MZ and DZ cross-twin/cross-age correlations are similar, shared environmental influences are expected to play a role in the stability over time. If MZ cross correlations are more than twice as large as DZ correlations, dominance effects may play a role in the continuity of cognitive ability. In this study, a model including influences of A, C, and E was tested, based on the twin correlations and cross correlations (see results-section).

Genetic modelling was performed using Mx (Neale et al., 2006), following several steps. The developmental pattern of verbal and nonverbal abilities from age 5 to 18 years was first examined in a Cholesky decomposition model. This approach decomposes the phenotypic relations into genetic, shared environmental and nonshared environmental contributions to the variance / covariance structure. All possible contributions are parameterised in the Cholesky decomposition; therefore it yields the best possible fit to the data. The model is descriptive rather than driven by any specific developmental hypothesis. However, it is useful to gain a first insight in what factors are important for the stability of verbal and nonverbal abilities. Furthermore it serves as a reference model to evaluate the fit of more parsimonious submodels. Based on the parameter estimates from the Cholesky decomposition, and on the findings from previous studies, several submodels were tested including two developmental mechanisms: the common factor model and the transmission model. In the common factor model (Figure 1a), one underlying verbal factor and one under-

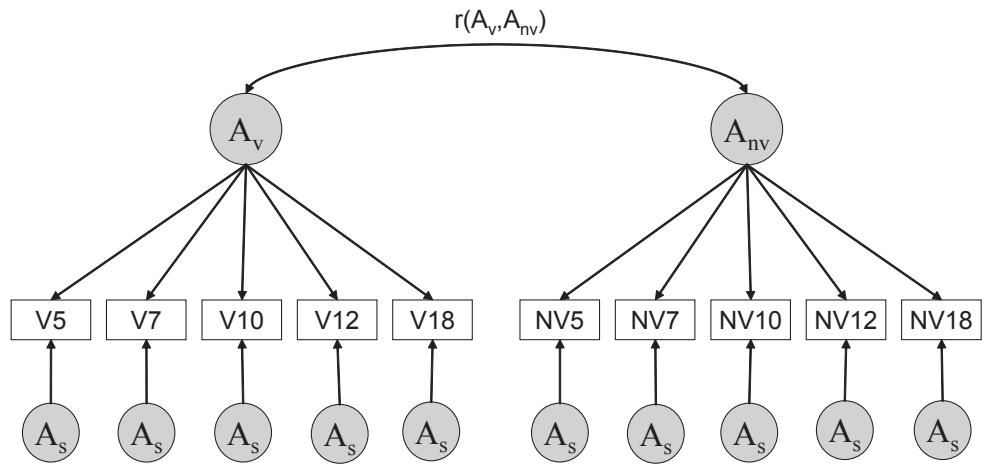

FIGURE 1a. Common factor model with age specific influences
Note, V5/NV5 = Verbal /Nonverbal abilities at age 5; Av/Anv = Common genetic factor exerting its influence on verbal /nonverbal abilities; As = age specific genetic influences. Path diagram is shown for additive genetic effects. The same model was also tested for shared environmental effects.

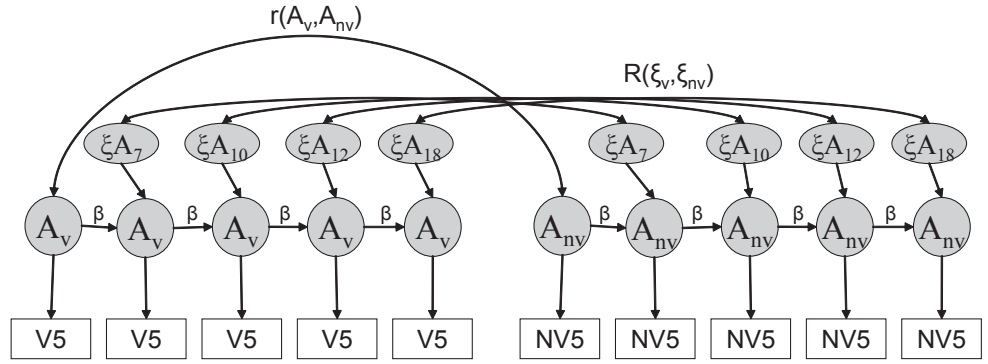

FIGURE 1b. Transmission model
Note, V5/NV5 = Verbal /Nonverbal abilities at age 5; Av/Anv = Genetic influences on verbal /nonverbal abilities; ξA = genetic innovation; β = genetic transmission to subsequent time point.

lying nonverbal factor are specified. These factors imply a continuous influence over time from the time of onset. The common verbal and nonverbal factors are allowed to correlate with each other. The transmission model (Figure 1b) represents a first-order autoregressive process. The covariances among the five measurement occasions are specified by the transmission of these effects to subsequent ages. Apart from the influences from prior time points, an innovation term unique to each measurement occasion can affect the variance. The total variance at each time point is the sum of the innovation effect and the age-to-age carry-over effect. Transmission and innovation factors are specified separately for verbal and nonverbal abilities. The genetic effects at age 5 and the innovation effects at subsequent time points on both abilities are allowed to correlate with each other at each time point.

The fit of the different developmental models and more parsimonious submodels was evaluated against the Cholesky model using χ^2 tests. The likelihood ratio test, which is the difference between minus twice the log likelihoods (-2 LL) of the two nested models under investigation, is distributed as a χ^2. The degrees of freedom (df) are given by the difference in the number of parameters estimated in the two models. A high increase in χ^2 against a low gain of degrees of freedom denotes a worse fit of the submodel compared to the full model. The most parsimonious model, with still a limited χ^2, is chosen as the best fitting model. As the transmission model and the common factor model are not nested, it is impossible to use the χ^2 test to evaluate which model fits better. To select the best model, Akaike's information criterion (AIC=χ^2 – 2df) was computed. The model with the lowest AIC reflects the best balance between goodness of fit and parsimony. The best fitting parsimonious model was used to derive estimates of genetic, shared environmental and nonshared environmental effects on the variances and covariances of verbal and nonverbal abilities.

Results

The descriptives of verbal and nonverbal abilities at all five time points are given in Table 1. All variables were approximately normal distributed at all ages. Mean differences due to birth order or zygosity of the twins were absent. Mean verbal IQ scores were higher in boys than in girls (χ^2=14.919, df=5, p=.011). The direction of the sex difference in mean nonverbal IQ (χ^2=13.836, df=5, p=.016) varied per age group. In the genetic model fitting the means were specified separately for boys and girls, to account for the sex differences in the mean.

Table 2 summarises the phenotypic correlations for verbal (above diagonal) and nonverbal (below diagonal) abilities across time. Both verbal and nonverbal abili-

TABLE 1. Descriptives of the verbal and nonverbal IQ scores for all subjects at all time points (age 5, 7, 10, 12, and 18 years).

All twins	N	Mean	SD
Verbal IQ age 5	415	103.79	13.47
Nonverbal IQ age 5	418	101.17	13.75
Verbal IQ age 7	382	97.68	14.22
Nonverbal IQ age 7	384	107.25	16.10
Verbal IQ age 10	392	103.09	14.90
Nonverbal IQ age 10	394	108.68	16.33
Verbal IQ age 12	381	97.33	12.96
Nonverbal IQ age 12	383	103.10	14.05
Verbal IQ age 18	365	101.02	19.36
Nonverbal IQ age 18	364	107.12	17.01

ties show substantial stability over time; the phenotypic correlation over a 13-year time interval is .51 for verbal abilities and .47 for nonverbal abilities. However, the phenotypic correlations decrease as the time intervals get larger. The last column in Table 2 gives the correlations between verbal and nonverbal abilities. As can be seen, these correlations increase with age. In early childhood verbal and non-verbal cognitive abilities are still largely independent (r=.33 - .35 at age 5, 7, and 10 years), and become increasingly more correlated in later stages of development (r=.58 at age 12, and .57 at age 18).

Table 3 shows the twin correlations for the five zygosity groups estimated separately at each age. At all ages, and for both verbal and nonverbal abilities, the MZ correlations are higher than the DZ correlations, indicating genetic influences. The only exception to this pattern is verbal IQ at age 7, when MZ correlations are of the same magnitude as the DZ correlations. Apart from age 18, MZ correlations are not twice

TABLE 2. Longitudinal phenotypic correlations for verbal abilities (above diagonal) and nonverbal abilities (below diagonal) and the cross-sectional correlations between verbal and nonverbal abilities at age 5, 7, 10, 12 and 18 years.

age	5	7	10	12	18	$R_{v\text{-}nv}$
5	-	.64	.61	.55	.51	.33
7	.58	-	.61	.56	.55	.35
10	.57	.71	-	.68	.67	.35
12	.54	.62	.66	-	.80	.58
18	.47	.57	.63	.61	-	.57

TABLE 3. Twin correlations for verbal and nonverbal abilities in all zygosity groups.

	Verbal abilities					Nonverbal abilities				
age	5	7	10	12	18	5	7	10	12	18
MZM	.75	.56	.84	.86	.88	.71	.60	.73	.85	.76
DZM	.62	.57	.51	.65	.42	.46	.38	.58	.48	.31
MZF	.81	.70	.80	.87	.81	.56	.71	.70	.77	.74
DZF	.65	.73	.33	.59	.52	.43	.30	.40	.57	.44
DOS	.63	.55	.50	.56	.38	.65	.53	.42	.49	.57
All MZ	.77	.61	.82	.86	.83	.61	.68	.71	.81	.73
All DZ	.59	.58	.42	.59	.41	.49	.42	.45	.45	.39

Note: MZM = monozygotic male twin pairs; DZM = dizygotic male twin pairs; MZF = monozygotic female twin pairs; DZF = dizygotic female twin pairs; DOS = dizygotic opposite sex twin pairs; All MZ = all monozygotic twin pairs; All DZ = all dizygotic twin pairs.

as high as DZ correlations, suggesting that shared environmental influences also play a role in familial resemblances. For both verbal and nonverbal abilities, the difference between MZ and DZ correlations tends to increase with age, suggesting that genetic influences become increasingly important with age. These patterns of correlations also suggest decreasing effects of the shared environment over time. Twin correlations in twins of opposite sex are similar to dizygotic same sex twins, yielding no indication that sex-specific genes are of importance. The significance of sex differences in twin correlations was tested for both verbal and nonverbal IQ. Constraining MZ and DZ correlations to be the same across the sexes did not significantly worsen the model fit, neither for verbal abilities (χ^2=130.637, df=120, p=.239) nor for nonverbal abilities (χ^2=139.144, df=120, p=.112). Therefore in subsequent modelling, data from male, female and opposite sex twins were pooled into 2 groups (MZ and DZ twins).

Table 4 gives the MZ and DZ cross-twin/cross-age correlations for verbal and nonverbal abilities. MZ correlations are higher than DZ correlations, especially for nonverbal abilities, indicating genetic influence on stability. For verbal abilities, the MZ cross correlations are not twice as high as the DZ cross correlations, suggesting that for the stability of these cognitive abilities, shared environmental influences may also be of importance. Table 4 also shows the cross-twin/cross-trait correlation

TABLE 4. Cross-twin/cross-age correlations over time for verbal and nonverbal abilities in monozygotic (MZ, above diagonal) and dizygotic (DZ, below diagonal) twins, and the cross-twin/cross-trait correlations between verbal and nonverbal abilities in MZ (first number) and DZ (second number) twins at age 5, 7, 10, 12 and 18 years.

	Verbal abilities						Nonverbal abilities					Cross $R_{v\text{-}nv}$
age	5	7	10	12	18	age	5	7	10	12	18	MZ/DZ
5	-	.59	.58	.55	.42	5	-	.54	.57	.56	.58	.32/.32
7	.48	-	.61	.55	.50	7	.37	-	.66	.60	.62	.28/.21
10	.40	.40	-	.65	.65	10	.33	.35	-	.63	.65	.32/.27
12	.41	.37	.41	-	.79	12	.37	.36	.38	-	.65	.53/.37
18	.36	.33	.35	.43	-	18	.23	.25	.34	.37	-	.53/.28

between verbal and nonverbal abilities at each time point. Apart from the first measurement occasion, MZ cross correlations are higher than DZ cross correlations, suggesting genetic effects on the overlap between verbal and nonverbal IQ.

A series of developmental models was fitted to the data on verbal and nonverbal IQ (verbal abilities at age 5, 7, 10, 12, and 18 years of age, and nonverbal abilities at the same 5 time points). Table 5 gives the model fitting statistics for the Cholesky decomposition and the more parsimonious submodels. The parameter estimates from the Cholesky decomposition were inspected to get a first impression of the importance of the influence of A, C, and E to the variances and covariances between measures and between twins. The Cholesky decomposition was used as the reference model to evaluate the fit of developmental models, incorporating different mechanisms for A, C, and E.

The parameter estimates based on the Cholesky decomposition are given in Table 6. These estimates, together with results from previous studies (Bartels et al., 2002; Petrill et al., 2004; Rietveld et al., 2003) both suggested that nonshared environmental influences are only of importance for explaining age specific variance in cognitive abilities, and do not have a significant role in explaining stability. Therefore, a model with solely age specific effects of the nonshared environment was applied. The parameters describing the shared environmental influences in the Cholesky decomposition showed the highest loadings on the first factor, and relatively low loadings on the other factors. Furthermore, the loadings were higher for verbal than for non-

TABLE 5. Model fitting results for multivariate longitudinal analyses of verbal and nonverbal abilities.

model	-2LL	df	Cpm	χ^2	p	AIC
1. ACE Cholesky	29270.163	3693				
2. ACE A common + age specific C common + age specific E age specific only	29403.336	3806	1	133.173	.095	-92.827
3. ACE A transmission C common + age specific E age specific only	29392.248	3804	1	122.085	.222	-99.915
4. ACE A transmission C common verbal only C age specific verbal + nonverbal E age specific only	29402.021	3810	3	9.773	.135	
5. ACE A transmission C common verbal only C age specific verbal only E age specific only	29405.304	3815	4	3.283	.656	
6. ACE A transmission C common verbal only, no age 18 C age specific verbal only E age specific only	29406.790	3816	5	1.486	.223	
7. ACE A transmission C common verbal only, no age 18, 12 C age specific verbal only E age specific only	29408.735	3817	6	1.945	.163	
8. ACE A transmission C common verbal only, no age 18, 12, 10 C age specific verbal only E age specific only	29427.400	3818	7	18.665	<.001	
9. ACE A transmission C common verbal only, no age 18, 12 C age specific verbal only, no age 18 E age specific only	**29408.735**	**3818**	**7**	**0**	**1.00**	
10. ACE A transmission C common verbal only, no age 18, 12 C age specific verbal only, no age 18, 12 E age specific only	29413.496	3819	9	4.761	.029	

Note: -2LL = -2 log likelihood; df = degrees of freedom; cpm = compared to model

TABLE 6. Parameter estimates for additive genetic, shared environmental, and nonshared environmental influences as derived from the Cholesky decomposition.

		Verbal abilities					Nonverbal abilities				
	age	5	7	10	12	18	5	7	10	12	18
	Cholesky parameter estimates additive genetic effects										
Verbal	5	8.05									
	7	6.21	4.37								
	10	8.17	7.62	3.62							
	12	6.20	5.77	-1.50	4.10						
	18	7.22	12.18	-1.77	8.01	2.02					
Nonverbal	5	1.74	4.55	-4.43	.02	2.50	2.77				
	7	3.76	5.05	-5.83	1.60	5.80	5.81	.01			
	10	3.39	5.29	-6.40	2.89	5.52	5.27	.01	.00		
	12	4.18	5.49	-4.36	.04	.71	7.94	.03	.00	.01	
	18	2.80	8.31	-7.77	.01	4.39	5.95	.01	.00	.00	.00
	Cholesky parameter estimates shared environmental effects										
Verbal	5	8.62									
	7	7.53	4.92								
	10	5.52	.34	2.72							
	12	5.19	-.20	3.43	4.05						
	18	6.56	-.67	1.68	1.53	1.13					
Nonverbal	5	5.46	-3.12	1.60	-1.99	-4.00	.04				
	7	3.14	.12	3.11	.76	-3.94	.05	.00			
	10	3.73	-2.24	4.95	-1.07	.13	.00	.00	.00		
	12	4.10	-2.77	2.34	1.07	-.73	.02	.00	.00	.00	
	18	4.13	-1.85	1.09	.74	2.52	-.02	.00	.00	.00	.00
	Cholesky parameter estimates nonshared environmental effects										
Verbal	5	6.40									
	7	1.28	8.09								
	10	.97	-.03	6.44							
	12	.02	.29	.56	4.80						
	18	2.59	.88	.59	1.55	6.99					
Nonverbal	5	-.03	-.13	-.44	-.15	-.29	8.21				
	7	-.39	1.34	1.04	-.16	2.87	.95	8.37			
	10	-.89	.87	.90	.37	.79	.73	1.43	8.41		
	12	-.45	.57	.34	1.44	.30	-.44	-.02	.40	6.30	
	18	-1.19	1.07	.81	.89	2.52	-.45	-.59	-.47	-2.37	7.94

verbal abilities. This pattern suggests a common factor structure, with highest loadings on verbal IQ. Prior studies (Bartels et al., 2002; Bishop et al., 2003; Rietveld et al., 2003) also indicated that, if of importance, shared environmental influences would exert their effects via a common factor structure. Since one previous study (Bartels et al., 2002) in the same sample as the current study also found significant age specific effects of the shared environment, these effects were specified as well. The previous literature on the developmental mechanism underlying genetic influences is less clear-cut. Some studies report a transmission pattern (Cardon et al., 1992; Cardon, 1994; Rietveld et al., 2003), others found a common factor model (Bartels et al., 2002; Petrill et al., 2004a), or a combination of these models (Bishop et al., 2003). In our data the Cholesky decomposition did not give a clear indication for a transmission structure (i.e. decreasing factor loadings with increasing time intervals) or a common factor developmental pattern (i.e. high loadings on one factor). Therefore, both a transmission model and a common factor model including age specific effects were fitted to the data.

To summarise, two models were evaluated. The first model included a common factor structure together with age specific influences for A and C, combined with only age specific influences for E (model 2 in Table 5). In the second model, genetic effects were modelled in a transmission structure, shared environmental effects in a common factor structure including age specific effects, while nonshared environmental influences were again specified to only have age specific effects (model 3 in Table 5). Application of these submodels did not result in a significant deterioration of the fit compared to the Cholesky decomposition (model 2: χ^2=133.173, df=113, p=.095; model 3: χ^2=122.085, df=111, p=.222). To evaluate whether model 2 or model 3 showed a better fit to the data, AIC's were compared. Since the AIC was lowest for the model including a transmission structure for additive genetic influences (model 3), this model was chosen as the best model.

We next tested the significance of the loadings on the shared environmental factor separately for verbal abilities and nonverbal abilities. Constraining the factor loadings on the nonverbal common factor to be zero (model 4) did not lead to a significant drop in model fit (χ^2=9.773, df=6, p=.135). Age specific shared environmental influences on nonverbal abilities were not significant either (model 5, χ^2=3.283, df=5, p=.656). These results indicate that all shared environmental influences on nonverbal abilities could be omitted. Subsequently, the significance of the shared environmental common factor on verbal abilities was tested. Since previous studies reported diminishing influences of C with age, the significance of the loadings on the common factor at later time points was tested first. The role of common factorial C on verbal abilities appeared to be non-significant at age 18 (model 6, χ^2=1.486, df=1, p=.223), and at age 12 (model 7, χ^2=1.945, df=1, p=.163). The influence of the common factor

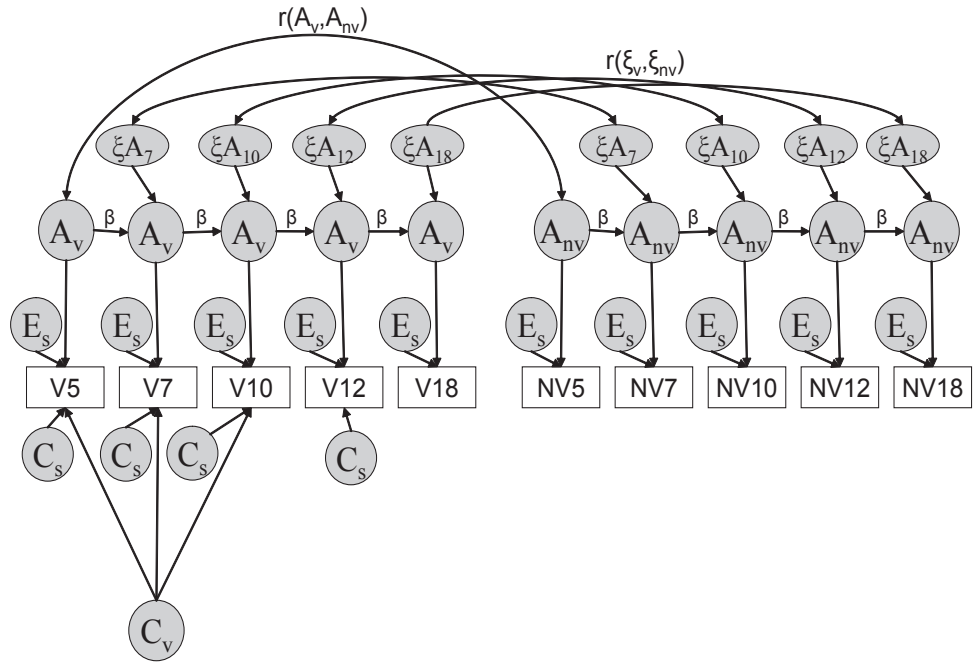

FIGURE 2. Path diagram of the best fitting model

Note, V5/NV5 = Verbal /Nonverbal abilities at age 5; Av/Anv = Genetic influences on verbal /nonverbal abilities; ξA = genetic innovation; β = genetic transmission to subsequent time point; Cv = shared environmental common factor influencing verbal abilities; Cs = age specific shared environmental influences; Es = age specific nonshared environmental influences.

on age 10 however was of significant importance. Constraining this factor loading to be zero resulted in a significant deterioration of the model fit (model 8, χ^2=18.665, df=1, p=<.001). Additionally, the significance of the shared environmental influences specific to each time point were tested. The age specific influences of C were not of significant importance at age 18 (model 9, χ^2=0.00, df=1, p=1.00), but were significant at age 12 (model 10, χ^2=4.761, df=1, p=.029). Taken together, the most parsimonious model with still acceptable fit (model 9 in Table 5; illustrated in Figure 2) was a model with i) a transmission structure for additive genetic influences; ii) a shared environmental common factor structure at age 5, 7, and 10 and time specific shared environmental influences at age 5, 7, 10 and 12 years for verbal abilities only; iii) nonshared environmental influences that only exert age specific effects. The covariance between verbal and nonverbal abilities is entirely accounted for by genetic effects, which were allowed to correlate at each time point.

Based on the best fitting model, the contributions of A, C, and E on the variance and covariance of verbal and nonverbal abilities were calculat-

TABLE 7. Contributions of additive genetic (A), shared (C) and nonshared (E) environmental influences to the variance in verbal and nonverbal abilities at age 5, 7, 10, 12 and 18 years, based on the best fitting model (95% confidence intervals in parentheses).

	Verbal abilities					Nonverbal abilities	
Variance	A transmission	C total	C common factor	C age specific	E age specific	A transmission	E age specific
5	.46 (.33-.59)	.28 (.16-.40)	.20	.08	.26 (.20-.34)	.64 (.54-.72)	.36 (.28-.46)
7	39 (.29-.49)	.28 (.18-.38)	.26	.02	.33 (.26-.42)	.68 (.61-.75)	.32 (.25-.39)
10	.56 (.48-.65)	.16 (.09-.24)	.08	.08	.28 (.21-.35)	.69 (.62-.76)	.31 .24-.38)
12	.80 (.73-.86)	.06 (.01-.12)	-	.06	.14 (.11-.19)	.74 (.65-81)	.26 (.19-.35)
18	.84 (.78-.88)	-	-	-	.16 (.12-.22)	.74 (.65-.80)	.26 (.20-.35)

ed. The contribution of genetic influences is given by the matrix formula:

$$A = (I-B)^{-1}*X*R*X'*((I-B)^{-1})'$$

where matrix B (dimension 10x10, for the 10 variables in the study) contains the genetic transmission parameters on its subdiagonal. The genetic innovation parameters are modelled in matrix X (a diagonal 10x10 matrix). Matrix R is a 10x10 correlation matrix, in which the 5 within-age correlations between the genetic innovations of verbal and nonverbal abilities are estimated. Matrix I (10x10) is an identity matrix. Likewise, the contribution of the shared environmental influences is obtained by the matrix formula:

$$C = Y*Y' + W*W'$$

where matrix Y (10x10) contains the loadings on the common factor (constrained to be zero for the nonverbal abilities, and for verbal abilities at age 12 and 18 years), and matrix W (10x10) contains the age specific C influences on the diagonal (constrained to be zero for the nonverbal abilities, and for verbal abilities at age 18). The contribution of the nonshared environmental influences is given by the matrix formula:

$$E = Z*Z'$$

where Z is a 10x10 diagonal matrix including the age specific influences of the nonshared environment.

The relative contribution of A, C, and E to the variance of verbal and nonverbal IQ are presented in Table 7. As indicated by the MZ and DZ twin correlations, additive genetic effects become increasingly important with age, especially for verbal abilities. The heritability of verbal abilities increases from 46% at age 5 to 84% at age

TABLE 8. Contributions of additive genetic (A), shared (C), and nonshared (E) environmental influences to the covariance in verbal and nonverbal abilities over time based on the best fitting model (95% confidence intervals in parentheses*).

	Verbal abilities			Nonverbal abilities	
Covariance	A transmission	C common factor	E age specific	A transmission	E age specific
5-7	.63 (.50-.77)	.37 (.23-.50)	-	1	-
5-10	.78 (.66-.89)	.22 (.11-.34)	-	1	-
5-12	1	-	-	1	-
5-18	1	-	-	1	-
7-10	.76 (.65-.86)	.24 (.14-.35)	-	1	-
7-12	1	-	-	1	-
7-18	1	-	-	1	-
10-12	1	-	-	1	-
10-18	1	-	-	1	-
12-18	1	-	-	1	-

* In the cases in which there is only one component specified to account for the covariance over time, this influence is per definition 100%. Therefore, the confidence intervals cannot be estimated in these cases.

18. Shared environmental influences also play a role in variance in childhood verbal IQ, but become insignificant in adolescence. Nonshared environmental influences seem to become slightly less important over time, but the confidence intervals for these effects at the different ages overlap. Shared environmental effects do not play a role in explaining variance in nonverbal abilities. The additive genetic effects become somewhat more important in explaining variance in nonverbal IQ at later stages of development, the heritability rises from 64% at age 5 to 74% at age 18. Table 8 shows the relative contribution of A, C, and E on the between-age covariance of verbal and nonverbal abilities. In early and middle childhood, the stability of verbal abilities is explained by both genetic and shared environmental influences. Between ages 5 and 10, shared environmental influences account for 22 – 37% of the covariance in verbal abilities. The remaining proportion of the covariance is explained by genetic effects that are transmitted to subsequent time points. At later stages of development (>10 years), shared environmental influences are no longer important, and the stability of verbal abilities is entirely accounted for by genetic effects. The nonshared environ-

TABLE 9. Genetic correlation (95% confidence intervals in parentheses) between verbal and nonverbal abilities at age 5, 7, 10, 12 and 18 years.

age	R_g
5	.62 (.54- .74)
7	.67 (.59- .79)
10	.57 (.52- .63)
12	.76 (.71- .81)
18	.73 (.69- .78)

mental effects on verbal abilities only exert age specific influences and do not contribute to the stability of verbal IQ. The stability of nonverbal abilities is entirely explained by genetic effects. The nonshared environmental effects are only age specific. Lastly, the genetic correlations between verbal and nonverbal abilities are given in Table 9. The overlap between verbal and nonverbal abilities is entirely explained by genetic effects. Similar to the phenotypic correlations between verbal and nonverbal abilities (see Table 2), the genetic correlation increases slightly with age, from .62 at age 5 to .73 at age 18.

Discussion

This study examined the genetic and environmental influences on verbal and nonverbal abilities between ages 5 and 18 years, investigated the developmental pattern underlying stability, and assessed the genetic correlation between verbal and nonverbal abilities at different ages. A sample of Dutch twin pairs was followed over a 13-year period, and cognitive tests were conducted when the twins were 5, 7, 10, 12, and 18 years old. These data showed that genetic effects on verbal IQ become increasingly important with age, whilst shared environmental influences decrease. For nonverbal IQ, genetic effects show a modest increase with age, and shared environmental influences could not be detected. The stability of verbal and nonverbal abilities is mainly accounted for by genetic effects that exert their influence via a transmission structure. A shared environmental common factor structure is of moderate importance in explaining continuity in verbal abilities from age 5 to 10 years, but shared environmental influences are not important for stability in nonverbal abilities. Nonshared environmental influences exerted time specific influences only, and did not influence the stability of cognitive abilities. The overlap between verbal and nonverbal abilities is entirely accounted for by genetic effects, and this overlap increases slightly with age.

Genetic and environmental influences at different time points

The increase of genetic effects on verbal and nonverbal abilities with age is in accordance with findings from previous cross sectional and longitudinal studies into cognitive development (Bartels et al., 2002; Bishop et al., 2003; Deary et al., 2006; Petrill et al., 2004a; Plomin & Spinath, 2004; Wilson, 1983). However, the current study is the first to cover cognitive development from childhood into young adulthood, and separates cognitive development into verbal and nonverbal abilities. We found the heritability of verbal IQ to increase from 46% at age 5 to 84% at age 18 years. For nonverbal IQ, genetic effects explained 64% of the variance at age 5. This proportion increased to 74% in young adulthood, but the confidence intervals of the heritability estimates at the different ages overlap, and are therefore not significantly different from each other. The heritability estimates found at age 18 years are similar to the estimates reported in previous studies in young and middle-aged adults (Posthuma et al., 2001; Rijsdijk et al., 2002) that found a heritability of 84-85% for VIQ and of 68-69% for PIQ.

Shared environmental influences were found to only be of importance for individual differences in verbal abilities in childhood. The shared environment was not of importance for explaining variance in nonverbal IQ. In studies into the development of general cognitive abilities, some reported significant influences of shared environmental effects (Bartels et al., 2002; Bishop et al., 2003; Rietveld et al., 2003) whilst others failed to find significant effects of the shared environment (Petrill et al., 2004a). The results of our study suggests that shared environmental influences are mainly important for verbal IQ, and less so for nonverbal aspects of cognitive performance. In accordance with previous studies (Bartels et al., 2002; Bishop et al., 2003), we found the shared environmental effects to decrease with age. Whilst shared environmental influences accounted for 28% of the variance at age 5 and age 7, these influences decreased to 6% at age 12 and became insignificant at age 18.

Longitudinal analyses: genetic and environmental effects on stability

Verbal and nonverbal abilities were found to be highly stable over time. Over a 13-year time interval the phenotypic correlations were around .50. The stability of nonverbal IQ was entirely accounted for by genetic effects. Stability of verbal IQ showed a moderate influence of shared environmental factors in early and middle childhood, but was entirely explained by genes in later phases of development. The major genetic effects on stability of cognitive performance are in agreement with findings from prior longitudinal studies (Bartels et al., 2002; Petrill et al., 2004a; Rietveld et al., 2003). Previous studies were inconclusive about the developmental mechanism underlying stability of cognitive development. Some studies found that a common factor structure gave a better description of the stability (Bartels et al., 2002; Petrill

et al., 2004a), whilst others reported a transmission structure (Cardon et al., 1992; Cardon, 1994; Rietveld et al., 2003) or a combination of both models (Bishop et al., 2003). Apart from Rietveld et al. (2003) and Cardon (1994), these previous studies examined general cognitive ability, and did not make a distinction into more specialised cognitive abilities. Similar to the latter two studies, in our project (which is a follow- up of the study of Rietveld et al., 2003) the underlying structure of genetic effects on stability was best described by a transmission model. This structure implies that, apart from substantial genetic effects that are carried over to continue to exert their influence on later time points, new genetic effects coming into play at subsequent time points are also of importance.

Shared environmental effects were found to have moderate effects on the stability of verbal abilities in early to middle childhood. These influences exerted their effects via a common factor structure loading at age 5, 7, and 10 years. In contrast, no shared environmental influences were found for nonverbal abilities. These findings indicate that children's development of verbal abilities is more prone to differences in the family environment than the development of nonverbal abilities. Factors such as socioeconomic status (SES) and parental education are highly stable and may underlie individual differences in verbal abilities in young children. Previous studies have shown that living in a high-SES neighbourhood is positively associated with IQ, verbal ability and reading ability in childhood and early adolescence, even when family characteristics associated with neighbourhood characteristics are taken into account (Leventhal & Brooks-Gunn, 2000). Various researchers have attempted to specify the characteristics of the home environment that may be related to cognitive abilities. The HOME (Caldwell & Bradley, 2003) is one of the most widely used measures of the family environment. A recent review of studies using the HOME throughout the world (Bradley & Corwyn, 2005) suggested that the positive influence of learning stimulation provided by the parents on the development of cognitive abilities are strongest in early childhood. This is in line with our finding of decreasing shared environmental effects in later phases of childhood. Unfortunately, most studies exploring the association between environmental influences and cognition do not control for genetic influences on this association. One exception to this is a study by Petrill and colleagues (2004b), who did a twin study in early childhood and examined whether SES and chaos in the home mediate the shared environmental variance associated with cognitive abilities. They found that both measures mediated a significant but modest proportion of the shared environment. However, these effects were found to be similar for verbal and nonverbal abilities, whilst we only found significant shared environmental influences for verbal abilities. Several behaviour genetics research groups have now started to include more precise measures of the

shared environment in their data collection. Only by collecting such indices, will it be possible to gain more insight into shared environmental influences.

In agreement with previous studies, nonshared environmental influences were found to only be of importance for effects specific to each time point and did not contribute to the continuity of cognitive abilities. Nonshared environmental influences are important in explaining why twins, and other children from the same family, are different from each other. Factors that may induce differences between twins and siblings could include traumatic experiences unshared with the co-twin, or consequences of an accident or illness. Also, if the children are in separate classes (as is the case for 37% of the twins, according to a large survey in 12-year-old twin pairs registered at the NTR), influences of the teacher will be nonshared. Within the Dutch primary school system, children normally change teacher each school year. The possible effects of a school teacher are thus likely to be age specific. Future studies should also include specific measures of nonshared environmental influences, in order to be able to examine the precise role of these effects on individual differences in cognitive abilities.

Genetic correlation between verbal and nonverbal abilities

Previous studies into the development of special cognitive abilities suggested that verbal and nonverbal abilities are largely genetically independent in early childhood, but become increasingly dependent in later phases of development (Petrill, 1997; Petrill et al., 2001; Price et al., 2000; Rijsdijk et al., 2002). The phenotypic association between verbal and nonverbal IQ in our study showed a slight increase over time, from .33 at age 5 to .57 at age 18. The overlap between verbal and nonverbal IQ was entirely accounted for by genetic effects. Following the increase in phenotypic correlation, the genetic correlation between verbal and nonverbal abilities also increased over time, from .62 at age 5 to .76 at age 12 and .73 at age 18. The genetic correlations found in the current study are larger than the correlations reported by Rietveld et al (2003), who used the same study sample when the twins were 5, 7, and 10 years old. In Rietveld's study, genetic modelling was done on the six subtest scores of the RAKIT. In the current study we used composite verbal and nonverbal IQ scores instead of subtest scores. Additionally, Rietveld et al. examined the significance of shared environmental influences on all subtests together and did not test whether the influences may only be significant for verbal subtests. Therefore, in their model, part of the overlap between verbal and nonverbal abilities was accounted for by shared environmental effects. As the influence of shared environmental effects was found to be non-significant for nonverbal abilities in the current study, the covariance between verbal and nonverbal IQ was entirely explained by genetic effects (i.e. the bivariate heritability between verbal and nonverbal ability was 100%). Therefore, the genetic

correlation (which is the standardised bivariate heritability) is higher in the current study than in the study by Rietveld et al.

The genetic correlation found in our study at age 18 was similar to the correlation reported by Posthuma et al. (2001) in young and middle-aged adults. They found a genetic correlation of .65 between VIQ and PIQ. Studying several specific cognitive abilities in the CAP project, Alarcón et al. found an average genetic correlation of .48 in 12-year-olds (Alarcón et al., 1998) and of .52 in 16-year-olds (Alarcón et al., 1999). In 2-year-old twins, Price et al (2000) reported a genetic correlation of .30. These results, together with the findings from our study, suggest that the genetic correlation between specific cognitive abilities increases with age. A strong genetic correlation is often conceived as evidence for a biological basis of g and for the existence of generalist genes (Kovas & Plomin, 2006; Petrill, 1997; Plomin & Spinath, 2002). Our findings supports this hypothesis. However, the genetic correlations are significantly different from one at all ages, indicating that there is also substantial genetic variance in verbal abilities that is unshared with nonverbal abilities, and vice versa.

Further considerations

To our knowledge, this is the first twin study reporting on the development of verbal and nonverbal abilities spanning childhood to young adulthood. Studying cognitive development over a broad time span necessitates different measurements per age group, simply because the development of cognition over such a long time cannot be captured by one test. One of the difficulties that come along with this is that no distinction can be made between true changes in development and changes due to different measurement instruments. In our study, we used the same test at the first three measurement occasions, namely the RAKIT. On the fourth and the fifth measurement occasion the WISC-R and the WAIS-III was used. The concurrent validity of the RAKIT and the WISC-R is .86 (Pijl et al., 1984). The correlation between VIQ and PIQ scores as measured with the WISC compared to the WAIS are respectively .89 and .76 (Wechsler, 1981). Based on this, we feel that it is likely that the patterns reported here reflect true development.

One of the challenges of longitudinal studies is drop out bias. Because of its longitudinal nature, this study had to deal with drop-outs over the years. Up to the fourth measurement occasion, more than 90% of the original sample still participated. As the twins had reached adulthood by the fifth measurement occasion, by then the choice of participation was no longer made by the parents. Also, at this age many twins had full time jobs or were enrolled in a study programme. For many families, lack of time or difficulties to take leave was the prime reason to no longer take part. At age 18 the participation rate decreased to 58% and new families were recruited in order to obtain a sufficient sample size. Comparison of the subjects who continued

participation and the families who dropped out revealed higher cognitive ability scores at age 5 in the subjects who continued participation. To correct for any bias this may cause on parameter estimates, the data analyses were performed on the raw data, so that longitudinally incomplete data could also be included (Little & Rubin, 2002).

We did not find sex differences in MZ and DZ twin resemblance for verbal and nonverbal IQ. This is in accordance with nearly all genetic studies of cognition. For example, two twin family studies that included larger sample sizes in adults (Posthuma et al., 2001) and young children (Spinath et al., 2003) did not find substantial sex differences in MZ and DZ twin correlations for verbal and nonverbal IQ either.

Twin correlations and heritability estimates may be affected by possible effects of gene environment correlation or gene environment interaction (Plomin et al., 2001; Posthuma & De Geus, 2006; Van Leeuwen et al., 2007). Parents with above average cognitive abilities are likely to have children who show above average cognitive performance, based on genetic transmission. But these parents may also be more likely to provide cognitively stimulating materials and interactions with their children, and thus provide an advantageous environment (cultural transmission). If both phenomena are of importance these two forms of transmission induce a correlation between genes and environment. However, the recent study of Van Leeuwen et al. (2007) did not obtain evidence for cultural transmission in an independent sample of 9-year-old twins and their sibs. On the other hand, a certain genetic make-up could show differential expressions in different environments (gene environment interaction). For instance, a child with a genetic predisposition for above average cognitive performance may benefit more from an advantageous environment than a child with genetic vulnerability for low cognitive abilities. One approach to test for gene environment interaction is to examine the association between sum and difference scores in MZ twins (Jinks & Fulker, 1970). Sum scores of MZ twin pairs reflect familial effects (either genetic or shared environmental), while absolute differences within these pairs can only be caused by nonshared environmental effects. An association between these scores would suggest that (provided that shared environmental effects are absent), people with a certain genotype may be more vulnerable to nonshared environmental effects than people carrying other genetic variants (see also Van Leeuwen et al. (2007) for an application of this method). We explored this association for both verbal and nonverbal IQ at all 5 ages, resulting in 10 correlations. None of these were found to be significant, apart from the correlation for VIQ at age 7 ($r=.256$, $p=.024$) which is not significant after Bonferroni correction for multiple testing (Stevens, 1996). These results yield no indication for strong gene environment interaction on verbal and nonverbal IQ.

Our longitudinal data provide support for strong genetic effects on the continuity of verbal and nonverbal abilities from early childhood to young adulthood. Shared environmental effects are only of importance for verbal abilities, and these effects are limited to young and middle childhood. Nonshared environmental effects are of moderate importance in explaining individual differences in both verbal and nonverbal IQ, these effects are only age specific. These results add to the growing body of literature on the development of cognitive abilities that report that genetic effects are the driving force behind stability of cognitive abilities. Previous studies also agreed that environmental effects are mainly important in explaining why twins differ from each other, and are predominantly time specific. This latter finding is important, because it implies that, although environmental influences are of significant importance in explaining individual differences in cognitive abilities, these effects are mainly transient in nature. In our study, shared environmental effects were found to contribute significantly to the stability in cognitive abilities in early and middle childhood. This finding suggests that, even in a relatively egalitarian society like the Netherlands, variance in the family environment is of significant importance. However, the precise nature of the shared and nonshared environmental effects is still largely unknown. Fortunately, several research groups have now begun to a) measure specific genes by candidate gene studies, and b) include specific measures of the environment. These studies will greatly increase our knowledge about the precise role of genes and environment on the development of cognitive abilities.

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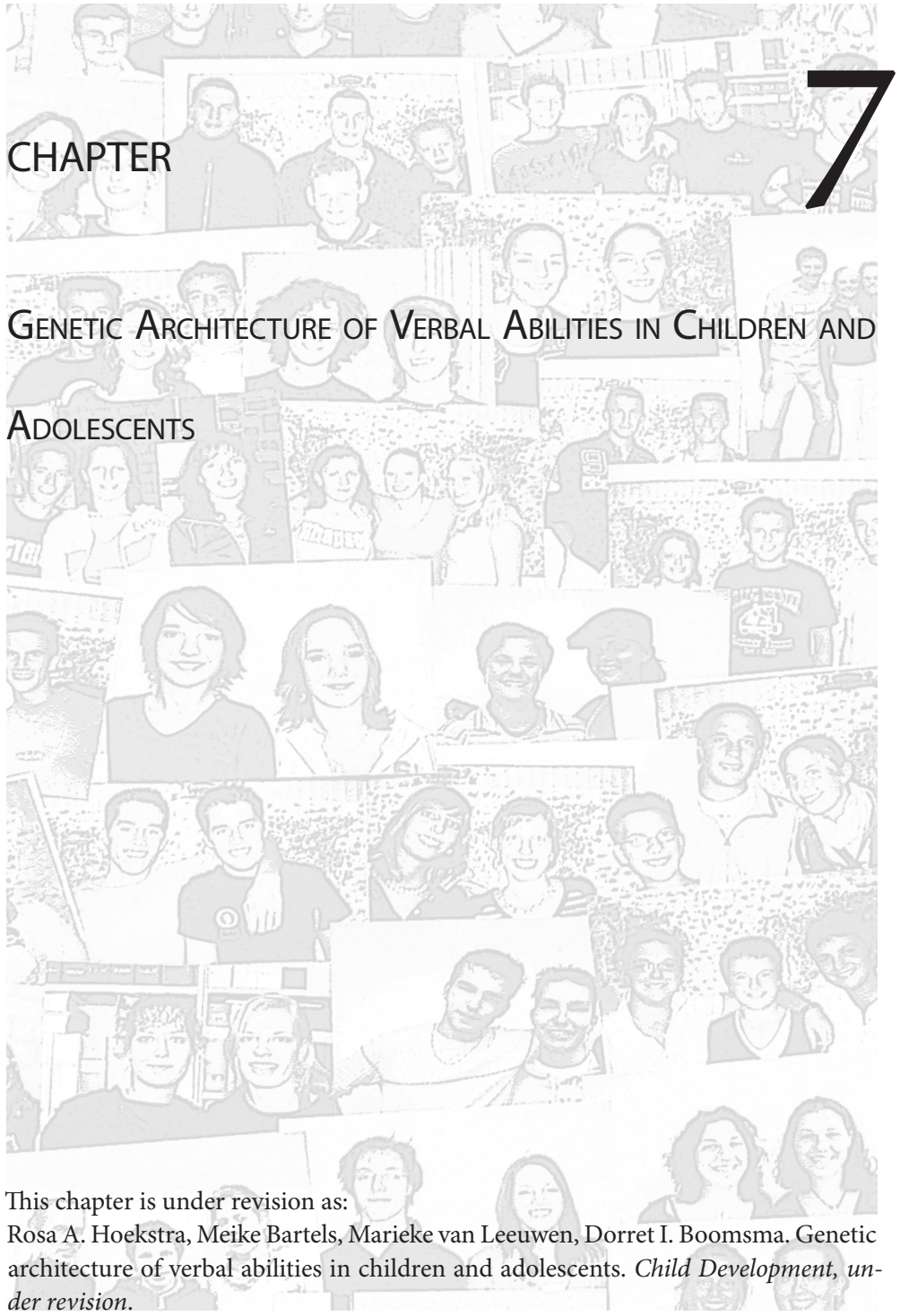

CHAPTER 7

Genetic Architecture of Verbal Abilities in Children and Adolescents

This chapter is under revision as:
Rosa A. Hoekstra, Meike Bartels, Marieke van Leeuwen, Dorret I. Boomsma. Genetic architecture of verbal abilities in children and adolescents. *Child Development, under revision*.

Abstract

The genetic aetiologies of variation in verbal abilities, including Wechsler verbal IQ (VIQ), verbal learning and memory, and letter and category fluency were examined in two independent samples of 9-year-old and 18-year-old twin pairs and their siblings. In both age groups, genetic effects were strong for VIQ, and moderate for verbal learning, memory, and fluency. Remarkably, all familial resemblance was explained by shared genes without any influence of shared environment. There was significant covariance among the tests, which was mainly explained by genetic effects, both in middle childhood and in late adolescence. The genetic correlations between the verbal abilities were somewhat stronger in the adolescent cohort, suggesting increasing genetic unidimensionality with age.

Introduction

The development of verbal abilities form a crucial part of a child's maturation process. Verbal abilities are key components for acquiring language, and learning how to read and write. Moreover, verbal abilities are needed for healthy social communicative functioning. A thorough understanding of the aetiology of individual differences in verbal abilities, of how different verbal abilities are related to one another, and about the development of these abilities over time, is therefore highly relevant. Adolescence is a critical period in cognitive and brain development. In this phase of life prominent developmental changes are seen in the prefrontal cortex and the limbic brain regions, including the hippocampus (Spear, 2000). These brain changes coincide with the development of increasingly elaborate cognitive abilities (Casey et al., 2000; Durston & Casey, 2006). Important aspects of verbal development such as verbal learning, verbal memory and verbal fluency continue to improve during late childhood (Sincoff & Sternberg, 1988; Van den Burg & Kingma, 1999) and adolescence (Clark et al., 2006; Levin et al., 1991), and these advances may be related to the changes in the brain (Durston & Casey, 2006; Gaillard et al., 2000). For instance, Gaillard et al. (2000) examined verbal fluency performance in a functional MRI study and found worse performance in children compared to young adults, together with less focal activation of the cortex area in children. Before understanding how brain maturation and cognition are related in adolescence, a thorough understanding of the cognitive development by itself in this time period is needed.

Verbal learning and memory involve the process of registration, storage, retention, and retrieval of verbal information (Lezak, 1995). This process can be assessed with tests such as the Rey's Auditory Verbal Learning Test (AVLT) or the California Verbal Learning Test (CVLT), which yield reliable indices of word learning and declarative memory (Lezak, 1995; Mulder et al., 1996; Van den Burg & Kingma, 1999). Tasks of verbal fluency measure the spontaneous generation of words and thus provide a test for language production and retrieval and access to the verbal lexicon. Moreover, verbal fluency tasks require cognitive flexibility (for rapidly shifting from one word to the next) as well as response inhibition and therefore also provide a test of executive functioning (Mitrushina et al., 1999). Girls tend to outperform boys both on tests of verbal learning and memory (Van den Burg & Kingma, 1999), and verbal fluency (Sincoff & Sternberg, 1988). Moreover, these tests are found to be positively related to verbal intelligence (Bolla et al., 1990). However, the precise nature of the overlap between general verbal intelligence, such as Wechsler verbal IQ scores (Wechsler, 1997; Wechsler, 2002), and more specialised verbal abilities such as verbal learning,

memory and fluency, is not well known. Moreover, little is known about the relation between these abilities over the course of development.

A powerful method to examine the aetiology of individual differences in cognitive abilities is the study of genetically related individuals. In the last two decades, twin, family and adoption studies have generated a wealth of knowledge about the genetic and environmental influences on various cognitive abilities, including verbal abilities. These studies show that genetic factors on general verbal intelligence increase over the life time, while the influence of shared environmental factors (influences from the environment that are shared between family members, and that thus make relatives more alike) decrease. Within the Twins Early Development Study (TEDS), variance in general verbal intelligence in infants was found to be strongly influenced by shared environmental effects, and only moderately (about 25%) by genetic effects (Price et al., 2000). The heritability increases to about 50% in middle childhood (Hoekstra et al., 2007) and to about 85% in adulthood (Hoekstra et al., 2007; Posthuma et al., 2001; Rijsdijk et al., 2002). In parallel, shared environmental influences decrease at later ages in childhood (Hoekstra et al., 2007) and become non-significant by adolescence (Hoekstra et al., 2007; Posthuma et al., 2001; Rijsdijk et al., 2002). The Colorado Adoption Project (CAP) has collected data on the development of cognitive abilities in adopted children and their adoptive and biological parents, and in non-adoptive families. The heritability of verbal abilities increased from 11% when the children were 4 years of age (Rice et al., 1989) to 24% and 26% when the children were 7 (Alarcón et al., 2003) and 12 years old (Alarcón et al., 1998; Alarcón et al., 2003), to 64% when the offspring was 16 years old (Alarcón et al., 1999).

The genetic and environmental influences on early childhood verbal short term memory and verbal fluency have been studied both in twins from TEDS, and in an international twin sample. In 4.5-year-old twins from TEDS, the heritability of both abilities was moderate (respectively 36% and 40%), while shared environmental influences were not significant (Kovas et al., 2005). In a combined sample of Australian, Scandinavian, and American children (Samuelsson et al., 2005), more prominent genetic influences were detected, both for verbal short term memory (57%) and for "rapid naming" (a measure thought to reflect verbal fluency; 64%). In this sample, modest influences of the shared environment were found for verbal memory (29%). In a study in 6-year-old twin pairs overrepresented with children at risk for language impairment, phonological short term memory was found to be under substantial (61%) genetic influence, while shared environmental effects were non-significant (Bishop et al., 2006). Studies in later phases of childhood have mainly focused on reading abilities and found significant genetic influence on teacher-rated reading achievement (Harlaar et al., 2007) and word recognition (Gayan & Olson, 2003). Studies into specific verbal abilities in adult samples are scarce. Swan et al.

(1999) examined verbal learning and memory in aging male twins and reported a heritability of 56%. In the same study sample, individual differences in verbal fluency were explained by moderate genetic influences (34%), a (statistically non-significant) shared environmental component (18%) and nonshared environmental influences (48%; Swan & Carmelli, 2002). In 18- to 25-year-old female twins, free recall of unrelated words and categorised words were both moderately heritable (respectively 55% and 38%; Volk et al., 2006). Ando et al. (2001) studied verbal and spatial working memory in a sample of 16- to 29-year-old twins and also included verbal and spatial ability scores on a standardised intelligence test. Verbal working memory was moderately heritable (43-48%), while general verbal ability was under strong genetic influence (65%). A common genetic factor explained 20-26% of the variance in the verbal tasks, suggesting that some of the genetic influences were general, while the rest of the variance was modality or test-specific.

To summarise, twin and adoption studies into general verbal intelligence indicate increasing genetic effects and decreasing shared environmental influences over the course of development. Studies on verbal memory and verbal fluency in early childhood and in adulthood suggest moderate to strong genetic influences, while shared environmental effects appear not to play a major role. It is less clear whether these different verbal abilities are influenced by common genetic factors or by ability specific genetic factors. Rather than studying the aetiology of individual differences separately for each variable under investigation (using univariate genetic analyses), the use of multivariate genetic analyses enables disentangling genetic and environmental effects on the covariance *between* traits. This way, it can be examined whether specific cognitive abilities show genetic or environmental overlap with other cognitive abilities. Moreover, the genetic correlation (r_g, the extent to which the actual genes influencing one trait correlate with the genes involved in another trait) between specific cognitive abilities can be studied.

Using the TEDS sample, measures of vocabulary and grammar were found to be substantially correlated in 2-year-old twins, both at the phenotypic (r_{ph}=.66) and the genetic (r_g=.61) level (Dale et al., 2000). In 4.5-year-old twins from TEDS, verbal category fluency correlated moderately with other measures of language development and the genetic correlations were substantial, ranging from .48 to .96 (Hayiou-Thomas et al., 2006). These results suggest common genetic influences on diverse aspects of language. Hohnen and Stevenson (1999) found a common genetic factor influencing literacy, phonological awareness and language in 6- to 7-year-old children, but also found evidence for test-specific genetic influence. An international study including 7-year-old twins (Byrne et al., 2007) found high phenotypic and genetic correlations between measures of word identification, reading comprehension and spelling. On the other hand, the correlations between these measures and

verbal learning were only moderate. All in all, the results from multivariate genetic studies into specific verbal and language abilities yield mixed results. The phenotypic and genetic correlations vary, depending on the age of the studied population and on the measures under study.

The current study aims to examine the relationship between general verbal ability, as measured with the Wechsler verbal intelligence scale, with more specialised verbal abilities such as verbal learning, memory and fluency. We investigated the genetic and environmental influences on the overlap between these abilities in two distinct phases of development: 1) middle childhood, by studying verbal abilities in 9-year-old twins and their siblings (also referred to as the "child cohort"); and in 2) late adolescence, by studying performance on verbal tests in 18-year-old twins and their siblings (the "adolescent cohort"). In most twin studies, only data of the twins are collected. Our study includes siblings of twins and we are able to test whether the covariance structure in the data is the same for all first-degree relatives (twins and siblings) or whether there is evidence for twin-specific effects.

Methods

Participants

All twin families were recruited via the Netherlands Twin register (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam (Bartels et al., 2007; Boomsma et al., 2006). The current project includes data from two longitudinal studies. The child cohort took part in a study into brain development and cognition in early puberty and consisted of a group of 112 9-year-old twin pairs (mean age 9.10 years, sd=0.10) and their 9- to14-year-old siblings (n=100, mean age 11.84 years, sd=1.16). Since these twin families also took part in an MRI study, there were some exclusion criteria, such as having a pacemaker or braces (Van Leeuwen et al., 2007a). Of all participating twin pairs, 23 were monozygotic males (MZM), 23 dizygotic males (DZM), 25 monozygotic females (MZF), 21 dizygotic females (DZF), and 20 were dizygotic twin pairs of opposite sex (DOS). For the same sex twin pairs, zygosity determination was based on DNA polymorphisms (88 twin pairs), or on questionnaire items (4 pairs; (Rietveld et al., 2000). There were 43 male and 57 female siblings. The adolescent cohort took part in a longitudinal study into the development of cognition and behavioural problems (see Hoekstra et al. (2007) and Bartels et al. (2002) for details on the longitudinal data collection). This group consisted of 186 families of 18-year-old twin pairs (mean age 18.18 years, sd=0.21) and their siblings (n=93, mean age=18.51 years, sd=4.73), and comprised 33 MZM pairs, 34 DZM pairs, 44 MZF pairs, 38 DZF pairs, and 37 DOS pairs. The zygosity of the same sex twin pairs was determined by DNA analyses (128 pairs), blood group polymorphisms (19 pairs), or questionnaire items (2 pairs; Rietveld et al., 2000). There were 46 male and 47 female siblings in this cohort. Both studies were approved by the Central Committee on Research Involving Human Subjects and the institutional review board of the VU University Amsterdam. Written informed consent was obtained from all participants who were 18 years of age or older, and from the parents of all underage participants.

Test procedures

In both study cohorts, the cognitive testing took place at the laboratory of the VU University. The cognitive test protocol in the child cohort started in the morning and took approximately 5 hours to complete, including breaks. The families of the adolescent cohort were seen by a paediatrician in the morning, who studied their physical development. These twin families completed the cognitive test protocol in the afternoon, which took about 3.5 hours, including a break. In both studies, children from the same family were tested on the same day in different rooms by experienced test administrators.

Measures

All participants in the child cohort and the siblings of the older cohort who were younger than 16 years, completed the full Wechsler Intelligence Scale for Children-Third edition (WISC-III; Wechsler, 2002). Verbal IQ (VIQ) scores were determined as the standardised score on 5 verbal subtests. The standardised scores were based on results of same-aged children from the Netherlands. All participants of 16 years of age or above (all part of the adolescent cohort) completed 11 subtests from the Wechsler Adult Intelligence Scale-Third edition (WAIS-III; Wechsler, 1997). Verbal IQ was calculated as the mean subtest score on 6 verbal subtests. The subtests were standardised for the appropriate age group, based on a population sample of same-aged subjects in the Netherlands.

In the child cohort, verbal learning and memory was assessed using the Dutch version of Rey's Auditory Verbal Learning Test (AVLT; Van den Burg & Kingma, 1999). In this task, a list of 15 unrelated, concrete nouns (e.g. bird; pencil) is presented over 5 learning trials and immediate recall is tested following each presentation. Twenty to thirty minutes after the fifth presentation, delayed recall is assessed. During the time interval, the children completed an emotion recognition task and an inspection time task. These tasks are nonverbal and non-memory related, and would therefore not interfere with the AVLT performance. Verbal learning was measured as the total number of correct words over the 5 learning trials. Verbal memory was assessed as the total number of words recalled after the delay. The test retest reliabil-

ity of the AVLT has been examined using parallel tests in 225 Dutch school children (Van den Burg & Kingma, 1999). Verbal learning and memory were found to be the most reliable measures of the task, with test-retest correlations of .70 (learning) and .62 (memory).

The 18-year-old twins and their siblings completed the Dutch adaptation of the California Verbal Learning Test (CVLT; Mulder et al., 1996). In this task, a list of 16 items, with four words from each of four categories (fruits; herbs and spices; clothing; tools) is presented. Similar to the procedure in the AVLT, the list is presented 5 times, and the participant is instructed to recall as many words as possible from the list following each presentation. Subsequently, a second list consisting of 16 items is presented (the interference list). After hearing the interference list, the subject is asked to recall as many items from the original list (short delay free recall), and to recall as many items from each of the semantic categories (cued recall). After a time interval of 20 minutes (in which the participants completed an emotion recognition task and an inspection time task), long delay free recall of the items is assessed, followed by an assessment of cued recall, and recognition of the items. Verbal learning was measured as the total number of recalled items on the 5 trials; memory was assessed as the total number of items recalled in the long delay free recall phase. The test-retest reliability of verbal learning in the Dutch CVLT has been examined in 17-74 year-old healthy subjects (Mulder et al., 1996) and was found to be .62 using a parallel test (n=384), and .58 when compared to the AVLT (n=108). Mulder et al. (1996) did not examine the test-retest reliability of memory. In a pilot study of our twin family project we examined the test-retest reliability of the CVLT in 29 healthy subjects aged 14-20 years, with an inter test interval of 2-3 weeks (see Van Leeuwen et al. (2007b) for details on the procedure of this pilot), and found a test-retest correlation of .86 for verbal learning, and of .66 for memory.

In both the child and adolescent cohort, tests of verbal fluency were administered. This test evaluates the spontaneous production of words starting with a certain letter (verbal fluency letters) or belonging to a certain semantic category (verbal fluency categories) within a limited amount of time. The participants completed 2 trials for both conditions, and were instructed to name as many words as possible in one minute starting with an R or a T (letter trials), or belonging to the category "animals" or "professions" (category trials). Within the letter trials, subjects were prohibited from saying proper nouns (e.g. Robert or Rotterdam) or saying the same word twice using a different ending (e.g. roast and roasted). To control for quantitative differences between trials within one condition (e.g. on average, the subjects named more animals than professions), Z-scores were calculated for each trial. Letter fluency was measured as the mean Z-score over the two letter trials; category fluency was calculated as the mean Z-score of the semantic trials. We also examined the test-retest reliability of verbal fluency in our pilot and found a test-retest correlation of .70 for letter fluency and of .93 for category fluency. To our knowledge, no other studies have assessed the test-retest reliability of verbal fluency in the Dutch language.

Statistical analyses

All analyses were carried out using structural equation modelling in the software package Mx (Neale et al., 2006). The significance of the effects of sex and age on the means of all verbal abilities was tested in a saturated model, which only specified that the multivariate data from family members could be correlated, but which did not impose any theoretical model on the covariance structure. The saturated model was used to estimate the correlations between all phenotypes in both age cohorts. It was tested whether the phenotypic covariance structure was the same in the two cohorts. The saturated model was also used to estimate twin and twin-sibling correlations, and twin and twin-sibling cross correlations (e.g. the correlation between VIQ in the oldest of the twin and verbal learning in the youngest of the twin). All data were analysed, including data of incomplete twin pairs and data of twins without an additional sibling using the raw data option in Mx.

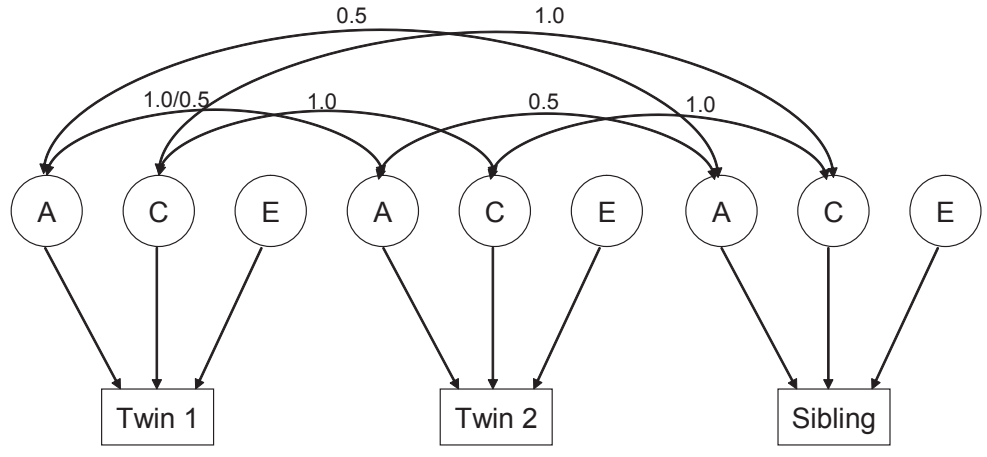

FIGURE 1. Univariate path diagram representing the contribution of additive genetic (A), shared environmental (C) and nonshared environmental (E) influences on the trait under investigation. The correlation of additive genetic factors is 1.0 in monozygotic twins, and, on average, 0.5 in dizygotic twins and between twins and siblings. The correlation of shared environmental effects is 1.0 between twins and between twins and siblings. Nonshared environmental effects represent influences unique to a family member and are thus uncorrelated.

Genetic modelling

Monozygotic (MZ) twins are (nearly) genetically identical, while dizygotic (DZ) twins and non-twin siblings share on average 50% of their segregating genes. Genetic model fitting of twin-sibling data allows for separation of the phenotypic variance into its genetic and environmental components (Figure 1). Additive genetic influences (A) result from the additive effects of alleles at each contributing genetic locus. Shared environmental influences (C) represent the environmental effects common to all offspring of the family. Nonshared environmental influences (E) are the effects of the environment that are not shared by the family members (including measurement error). Comparing the resemblance of MZ twins with the resemblance of DZ twins and twin-sibling pairs can give a first indication of what influences are important in explaining the variance in test performance. If MZ and first-degree relative correlations are similar, shared environmental influences are likely to be important. Higher MZ correlations compared to DZ and twin-sibling correlations indicate that genetic effects play a role (Boomsma et al., 2002). By comparing the resemblance of DZ twins with the resemblance between twins and their non-twin siblings, it is possible to test whether there is evidence for a twin specific environment. Higher DZ twin correlations compared to twin-sibling correlations would suggest such an effect. The use of multivariate genetic analyses enables the distinction of genetic and environmental effects on the covariance between traits. If the cross correlations in MZ twins and first-degree relatives are similar, shared environmental effects are important for explaining the covariance between the different verbal abilities. Higher MZ twin cross correlations compared to the cross correlation in first-degree relatives would indicate that genetic effects play a role in the overlap between verbal abilities.

The relative importance of the components A, C, and E was estimated using structural equation modelling in Mx (Neale et al., 2006). Genetic modelling was performed following several steps. The influences of A, C, and E on all verbal measures and on the overlap between the measures were first examined in a multivariate triangular or Cholesky decomposition (Neale & Cardon, 1992). This model decomposes the phenotypic relations into genetic, shared environmental and nonshared environmental contributions to the variance of a trait and to the covariance between traits. All possible contributions are parameterised in the Cholesky decomposition; therefore it yields the best possible fit to the data. It is useful to gain a first insight in what factors are important for the variance and covariance of verbal abilities. An ACE Cholesky decomposition was applied both to the data of the child cohort and to the data of the adolescent cohort. We then tested whether the genetic and shared environmental effects were of significant importance, by assessing the deterioration of the model fit after each component was dropped from the model. Next, it was tested whether the genetic influences on all tests could be described by a genetic common factor model (Figure 2). This model assumes that there is one underlying factor (e.g. a general verbal intelligence factor) that influences the individual differences in performance in each verbal test. To take the variance specific to each test into account, test specific genetic influences were also allowed in this model. Similarly, a common factor model including test specific influences was applied for the environmental influences. A good fit of this model would imply that there is one environmental factor that influences variance in performance on all verbal tests. Lastly, a model was tested in which the nonshared environmental influences were constrained to be test specific. Nonshared environmental influences were still allowed to covary between verbal learning and memory and between letter fluency and category fluency, as these variables were derived from the same test. After comparing the fit of these models, a best fitting most parsimonious model was established for the child cohort. Next, it was tested whether this model also fitted well to the data of the adolescent cohort.

The fit of the different submodels was evaluated against the Cholesky decomposition using likelihood ratio tests and Akaike's information criterion. The likelihood ratio, which is the difference between minus twice the log likelihoods (-2 LL) of two

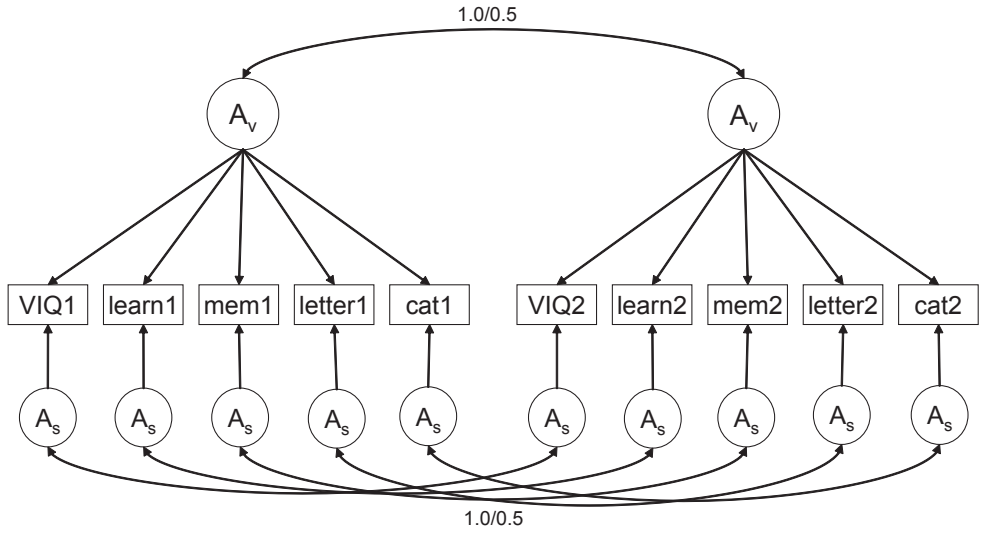

FIGURE 2. Path diagram depicting a common factor model including test specific influences. Path diagram shown for genetic effects, and for 2 family members only. This model can be expanded to include 3 family members, and can also be applied to shared environmental effects. Av = Common genetic factor exerting its influence on all verbal abilities. As = test specific genetic influences. Genetic effects correlate 1.0 between monozygotic twins, and on average 0.5 between dizygotic twins and twins and siblings. VIQ1/2 = verbal IQ family member 1/2; learn = verbal learning; mem = verbal memory; letter = letter fluency; cat = category fluency.

nested models, follows a χ^2 distribution. The degrees of freedom (df) are given by the difference in the number of parameters estimated in the two models. A high increase in χ^2 against a low gain of degrees of freedom denotes a worse fit of the submodel compared to the full model. The most parsimonious model, with still a limited χ^2, is chosen as the best fitting model. Akaike's information criterion (AIC=χ^2 – 2df) reflects the best balance between goodness of fit and parsimony with a model with the lowest AIC being the preferred model.

Results

The descriptives of the five different verbal abilities in both the child and the adolescent cohort are given in Table 1. A positive age effect (i.e. better performance with increasing age) was found for all specific verbal measures. The age effect was not tested for VIQ, as this measure is standardised for age. Females outperformed males on verbal learning and memory in both cohorts. Girls performed better than boys on the letter fluency task in the child cohort, but this sex difference was not significant in the adolescent cohort. Males showed superior performance on the VIQ scale in the adolescent cohort. In the genetic model fitting the means were corrected for these effects.

TABLE 1. Sample sizes, means, standard deviations, and effects of age and sex for the verbal ability measures in 9-year-old twins and their siblings (child cohort), and in 18-year-old twins and their siblings (adolescent cohort).

	N	Mean	SD	Sex effect	Age effect
Child cohort					
VIQ	324	102.35	15.47	-0.06	N/A
verbal learning	323	38.40	7.44	2.95*	2.61*
verbal memory	323	7.96	2.45	0.86*	0.61*
letter fluency	324	-0.39	0.77	0.28*	0.26*
category fluency	324	-0.28	0.72	0.11	0.25*
Adolescent cohort					
VIQ	457	105.61	18.77	-6.94*	N/A
verbal learning	456	56.35	7.99	2.72*	0.27*
verbal memory	455	12.26	2.37	0.63*	0.13*
letter fluency	456	-0.096	0.87	0.12	0.09*
category fluency	457	-0.02	0.84	-0.03	0.09*

Note: * p<.01. A negative sex effect denotes superior male performance; a positive effect represents better performance in females.

TABLE 2. Phenotypic correlations between verbal ability measures in the child cohort (above diagonal) and the adolescent cohort (below diagonal).

Task	VIQ	verbal learning	verbal memory	letter fluency	category fluency
VIQ	-	.28	.22	.33	.44
verbal learning	.48	-	.68	.26	.24
verbal memory	.45	.74	-	.21	.18
letter fluency	.40	.28	.20	-	.26
category fluency	.39	.37	.31	.47	-

Differences in variance and covariance due to zygosity or twin-sibling status were found to be absent in both cohorts. However, constraining the variance (χ^2=18.548, df=5, p=.002) and within person covariance (χ^2=33.257, df=10, p<.001) to be equal across the cohorts resulted in a significant deterioration of the fit. This indicates that the variance in the tests and the phenotypic covariance between the tests are significantly different in these two distinct phases of development. The phenotypic correlations between all measures are presented in Table 2, separately for the child (above diagonal) and the adolescent cohort (below diagonal). The phenotypic correlations are strongest between verbal learning and memory. Lower correlations are found between learning and memory and tests of verbal fluency. Overall, the phenotypic correlations are higher in the adolescent cohort (average r_{ph}=.41) than in the child cohort (average r_{ph}=.31).

Constraining the covariance matrices to be equal in DZ twins and in twin-siblings did not result in a deterioration of the fit of the saturated model, neither in the child cohort (χ^2=24.762, df=15, p=.053), nor in the adolescent cohort (χ^2=17.927, df=15, p=.267). This implies that the resemblance is similar in all first-degree relatives, and yields no evidence for a twin-specific environment. Table 3 displays the correlations in MZ twins (first figure on diagonal), and in DZ twins and twin-siblings (second

TABLE 3. Twin correlations and cross-correlations in MZ twins (below diagonal), and in DZ twins and twin-siblings (above diagonal) for five verbal ability tasks, in two cohorts.

Task	VIQ	verbal learning	verbal memory	letter fluency	category fluency
			Child cohort		
VIQ	.82/.47*	.18	.11	.22	.15
verbal learning	.24	.52/.18*	.19	.12	.07
verbal memory	.12	.41	.40/.22*	.14	.00
letter fluency	.34	.30	.15	.36/.23*	.05
category fluency	.36	.31	.11	-.03	.47/.01*
			Adolescent cohort		
VIQ	.84/.38*	.21	.20	.22	.18
verbal learning	.42	.34/.11*	.08	.11	.06
verbal memory	.39	.37	.47/.06*	.13	.05
letter fluency	.40	.28	.26	.51/.33*	.16
category fluency	.33	.21	.11	.37	.55/.19*

Note: *first figure correlation MZ twins, second figure correlation DZ twins and twin-siblings.

figure on diagonal). For all measures, MZ correlations are higher than DZ and twin-sibling correlations, indicating genetic influences. Moreover, apart from the correlations for verbal learning, the difference between the MZ twin and first-degree relative correlations is stronger in the older cohort than in the younger cohort. This suggests that the genetic influences on the variance in verbal abilities are stronger in late adolescence compared to middle childhood. In the child cohort, the MZ correlations for VIQ, verbal memory and letter fluency are not twice as high as the DZ and twin-sibling correlations, suggesting that shared environmental influences may also play a role. In both the child and the adolescent cohort, the MZ cross correlations (off-diagonal of Table 3) are higher than the DZ and twin-sibling cross correlations. This pattern suggests that the overlap between various measures of verbal abilities is

TABLE 4. Model fitting results for multivariate analyses of verbal abilities in the child and the adolescent cohort.

model		df	-2LL	cpm	χ^2	df	p	AIC
			Child cohort					
1.	ACE Cholesky	1559	7334.189					
2.	**AE Cholesky**	1574	7337.279	1	3.090	15	.999	-26.910
3.	CE Cholesky	1574	7371.181	1	36.992	15	.001	6.992
4.	AE A common factor + test specific	1579	7365.431	2	28.152	5	<.001	18.152
5.	AE E common factor + test specific	1579	7355.408	2	18.129	5	.003	10.129
6.	AE E test specific + correlated E between verbal learning and memory, and between verbal fluency tests	1582	7353.357	2	16.078	8	.041	.078
			Adolescent cohort					
1.	ACE Cholesky	2222	10600.738					
2.	**AE Cholesky**	2237	10603.722	1	2.984	15	.999	-27.016
3.	AE A common factor + test specific	2242	10620.318	2	16.596	5	.005	6.596
4.	AE E common factor + test specific	2242	10617.822	2	14.100	5	.015	4.100
5.	AE E test specific + correlated E between verbal learning and memory, and between verbal fluency tests	2245	10634.837	2	31.115	8	<.001	15.115

Note: df = degrees of freedom; -2LL = -2 log likelihood; cpm = compared to model; AIC = Akaike's Information Criterion; A = additive genetic influences; C = shared environmental influences; E = nonshared environmental influences.

influenced by genetic effects. In the child cohort, most MZ cross correlations are not twice as high as the DZ and twin-sibling cross correlations, suggesting that shared environmental influences may also explain part of the overlap between tests.

Table 4 gives the results of the model fitting for the Cholesky decomposition and the more parsimonious submodels. We started the model fitting procedure in the child cohort by testing the significance of the shared environmental influences (model 2 in Table 4) and the additive genetic influences (model 3) on the variance

TABLE 5. Contributions of additive genetic (A) and nonshared environmental (E) effects to the variance and covariance in verbal abilities, and the genetic correlations (r_g) between these abilities, in the child cohort. Estimates are based on the best fitting model (95% confidence interval in parentheses).

A

	VIQ	verbal learning	verbal memory	letter fluency	category fluency
VIQ	.82 (.72–.88)				
verbal learning	.94 (.61–1.00)	.46 (.30–.61)			
verbal memory	.59 (.00–.97)	.58 (.38–.76)	.42 (.25–.58)		
letter fluency	1.00 (.79–1.00)	.99 (.46–1.00)	.99 (.63–1.00)	.42 (.24–.58)	
category fluency	.71 (.46–.91)	.96 (.64–1.00)	.45 (.00–.97)	.19 (.00–.66)	.32 (.15–.51)

E

VIQ	.18 (.12–.28)				
verbal learning	.06 (.00–.39)	.54 (.39–.70)			
verbal memory	.41 (.03–1.00)	.42 (.24–.62)	.58 (.42–.75)		
letter fluency	.00 (.00–.21)	.01 (.00–.54)	.01 (.00–.37)	.58 (.42–.76)	
category fluency	.29 (.09–.54)	.04 (.00–.36)	.55 (.03–1.00)	.81 (.34–1.00)	.68 (.39–.85)

r_g

VIQ	-				
verbal learning	.40 (.19–.60)	-			
verbal memory	.20 (.00–.42)	.90 (.75–.99)	-		
letter fluency	.58 (.40–.79)	.51 (.20–.79)	.47 (.19–.76)	-	
category fluency	.64 (.41–.85)	.61 (.33–.85)	.24 (.00–.61)	.14 (.00–.54)	-

TABLE 6. Contributions of additive genetic (A) and nonshared environmental (E) effects to the variance and covariance in verbal abilities, and the genetic correlations (r_g) between these abilities, in the adolescent cohort. Estimates are based on the best fitting model (95% confidence interval in parentheses).

A

	VIQ	verbal learning	verbal memory	letter fluency	category fluency
VIQ	.84 (.77–.89)				
verbal learning	.92 (.76–1.00)	.28 (.15–.43)			
verbal memory	.90 (.72–1.00)	.37 (.21–.54)	.28 (.16–.44)		
letter fluency	.99 (.84–1.00)	.82 (.46–1.00)	.85 (.49–1.00)	.55 (.42–.66)	
category fluency	.88 (.69–1.00)	.41 (.09–.70)	.15 (.00–.50)	.70 (.47–.88)	.48 (.32–.62)

E

VIQ	.16 (.11–.23)				
verbal learning	.08 (.00–.24)	.72 (.57–.85)			
verbal memory	.10 (.00–.28)	.63 (.46–.79)	.72 (.56–.84)		
letter fluency	.01 (.00–.16)	.18 (.00–.54)	.15 (.00–.51)	.45 (.34–.58)	
category fluency	.12 (.00–.31)	.59 (.31–.91)	.85 (.50–1.00)	.30 (.11–.53)	.52 (.39–.68)

r_g

VIQ	-				
verbal learning	.90 (.70–1.00)	-			
verbal memory	.83 (.63–.98)	.95 (.78–.99)	-		
letter fluency	.60 (.46–.73)	.59 (.32–.85)	.47 (.22–.72)	-	
category fluency	.54 (.37–.70)	.42 (.10–.70)	.13 (.00–.44)	.65 (.47–.81)	-

and covariance in verbal abilities. Shared environmental effects were non-significant, dropping these effects from the model did not result in a deterioration of the model fit (χ^2=3.090, df=15, p=.999). The additive genetic influences were of significant importance (χ^2=36.992, df=15, p=.001). Subsequently it was tested whether the genetic effects on verbal abilities could be captured by a common factor model including test specific effects (model 4). Application of this model lead to a significant drop in model fit (χ^2=28.152, df=5, p<.001). Likewise, a model in which the nonshared environmental influences were constrained to a common factor including test specific effects (model 5) did not fit the data well (χ^2=18.129, df=5, p=.003). Lastly, a model with solely test specific influences of the nonshared environment, but permitting covariance between verbal learning and memory, and between letter fluency and category fluency (model 6) was fitted to the data of the child cohort. Application of this model resulted in a significant deterioration of the fit (χ^2=16.078, df=8, p=.041). All in all, the data of the child cohort were best described by a Cholesky decomposition including additive genetic and nonshared environmental effects (model 2). This model also had the lowest AIC-value, indicating that it showed the best balance between parsimony and model fit. Subsequently, the model fitting procedure was repeated for the adolescent cohort data. Similar to the results in the child cohort, the AE Cholesky model fitted the data best (χ^2=2.984, df=15, p=.999, AIC=-27.016).

The relative importance of additive genetic and nonshared environmental effects on the variance in each ability is given on the diagonal in Table 5 (child cohort) and Table 6 (adolescent cohort). In both age groups, the heritability was strongest for VIQ. Against expectation, the heritability of verbal learning and memory was stronger in the child compared to the adolescent cohort, although the confidence intervals overlap. The heritability estimates for verbal fluency were slightly higher in the adolescent cohort. The contributions of genes and environment on the covariance between the different verbal abilities are given on the subdiagonals of Table 5 and 6. In both cohorts, genetic effects account for most of the overlap between the tests, especially for the covariance between VIQ and the more specialised tests, and for the covariance between letter fluency and the other tests. In the child cohort, nonshared environmental effects are substantial for explaining the covariance between VIQ and memory, learning and memory, memory and category fluency, and between letter and category fluency. However the confidence intervals around these estimates are large. In the adolescent cohort, the nonshared environmental influences are substantial for the covariance between learning and memory, learning and category fluency, and between memory and category fluency. The genetic correlations between the different tests are given in the bottom of Table 5 and 6. On the whole, the genetic correlations are stronger in the adolescent cohort (average r_g over al tests=.61) compared to the child cohort (average r_g=.47). The genetic correlations in the adolescent cohort are

close to unity between VIQ, verbal learning, and verbal memory. The other genetic correlations are moderate, and range from .42 to .65, with one exception. The genetic correlation between memory and category fluency is estimated at .13, which is not significantly different from zero. In the child cohort, the genetic correlation is close to unity between verbal learning and memory. The other correlations are modest to moderate, ranging from .14 to .64.

Discussion

In this paper we report on the genetic and environmental influences on verbal abilities in two age cohorts. We studied the aetiology of the overlap in VIQ, verbal learning, verbal memory, and verbal fluency in two distinct phases of maturation: middle childhood and late adolescence. We found stronger correlations between the verbal tests in late adolescence, both at the phenotypic and the genetic level, suggesting progressing unidimensionality with age. We used an extended twin design and found that DZ twins did not resemble each other more closely than twin-sib pairs, yielding no indication for a twin-specific environment. In both cohorts, the individual differences in VIQ were strongly influenced by genetic effects, while the performance in more specific verbal abilities was under moderate genetic influence. The remaining variance was explained by nonshared environmental effects. Moreover, genetic effects were of major importance in explaining the overlap between the different verbal abilities, both in the child and the adolescent cohort. These results and their implications are discussed in more detail below.

Phenotypic correlations between verbal tasks in middle childhood and late adolescence

The within person variance and covariance on all tests was found to be significantly different in middle childhood than in late adolescence. Overall, the phenotypic correlations between the different verbal tests were stronger in adolescents, especially the correlations between VIQ and the more specific verbal measures (r ranging from .22 to .44 in the child cohort, and from .39 to .48 for the adolescent cohort). In a longitudinal cognition study, we found increasing phenotypic correlations between verbal and nonverbal abilities with age (Hoekstra et al., 2007). Together with the current data, these results suggest that cognitive abilities may be more generalised in a later stage of development. However, the adolescent cohort and child cohort participated in a somewhat different study protocol, and verbal learning and memory were assessed using slightly different tests (the AVLT in the child cohort vs. the CVLT in the adolescent cohort). Although previous studies indicated strong overlap between

AVLT and CVLT performance (Mulder et al., 1996; Stallings et al., 1995), with correlations close to the test-retest correlations of the CVLT itself (Mulder et al., 1996), we cannot exclude the possibility that these differences have affected the pattern of phenotypic correlations.

In both cohorts, the phenotypic correlations were strongest between verbal learning and memory. This is not surprising, as successful memory depends on successful learning, and both measures involve registration, storage, and retrieval of words. The phenotypic correlations between letter fluency and category fluency were relatively low (r=.26 in the child cohort and .47 in the adolescent cohort), indicating that these tasks tap different aspects of word fluency. Letter fluency requires phoneme analysis, while category fluency relies more heavily on semantic memory. Category fluency performance is shown to be superior in children who frequently use schemata to guide their recall (Sincoff & Sternberg, 1988). For instance, the subjects in our study could improve their performance on the "animal" trial of category fluency by thinking of all the animals that live in a zoo or on a farm. It is also possible to follow strategies in a letter fluency task (e.g. name words starting with the same consonants, such as *rep*tile and *rep*lication), but these strategies are not as obvious, and less often used.

Heritability of general vs. specific verbal abilities

Individual differences in general verbal abilities, as measured with the Wechsler VIQ, were found to be highly heritable, both in middle childhood and in late adolescence. The heritability estimate of 84% found in our sample of 18-year-old twins and their siblings is similar to heritability estimates of VIQ in other adult samples, that reported a heritability of 84 % (Rijsdijk et al., 2002) and 85% (Posthuma et al., 2001). The heritability estimate of 82% for VIQ in 9-year-old twins and their siblings is somewhat higher than the estimates reported in other studies in middle childhood (e.g. Hoekstra et al., 2007). However, the MZ and first degree relative correlations found in the child cohort (respectively r=.82 and r=.47) are very similar to the MZ and DZ twin correlations (rMZ=.82; rDZ=.42) found in our previous study in 10-year-old twins (Hoekstra et al., 2007). The latter study incorporated a longitudinal design, and found evidence for shared environmental influences in early and middle childhood. Therefore, the different heritability estimates in these two studies are most likely due to the different designs used.

The genetic influences on the variance in verbal learning and memory were moderate in both cohorts. Against expectation, the point estimate of the genetic effects was higher in middle childhood (46% and 42% respectively) than in late adolescence (both 28%), although the confidence intervals overlap. The attenuated genetic effects in the adolescent cohort compared to the child cohort are most likely explained

by differences in the tests used to measure these abilities. In the child cohort, verbal learning and memory was assessed with the AVLT, in which a list of unrelated words is used. In the adolescent cohort, learning and memory performance was determined with the CVLT, including a list of words belonging to different categories. One previous twin study examined the heritability of uncategorised word learning versus categorised word learning (Volk et al., 2006), and found stronger genetic effects on uncategorised (55%) than on categorised learning (38%). This difference in heritability could explain why the heritability estimates for learning and memory were higher in the child cohort compared to the adolescent cohort. Genetic effects on letter and category fluency were moderate in the child cohort (42% and 32% respectively). Although confidence intervals overlap, the point estimates for the genetic effects on both measures were slightly higher in the adolescent cohort (55% and 48%), consistent with the increasing genetic influences on verbal abilities found in previous studies (Alarcón et al., 1998; Alarcón et al., 1999; Alarcón et al., 2003; Hoekstra et al., 2007).

The remaining variance in all tasks was explained by nonshared environmental effects. Shared environmental influences failed to be significant, both in the child and the adolescent cohort. The lack of shared environmental influences on individual differences in adult verbal abilities is in accordance with findings from previous studies (Ando et al., 2001; Posthuma et al., 2001; Rijsdijk et al., 2002; Swan et al., 1999; Swan & Carmelli, 2002; Volk et al., 2006). An earlier study on the heritability of Wechsler VIQ in middle childhood reported modest shared environmental influences (Hoekstra et al., 2007). Studies in early to middle childhood on verbal fluency and verbal memory reported modest (Samuelsson et al., 2005) or non-significant effects of the shared environment (Bishop et al., 2006; Kovas et al., 2005; Thompson et al., 1991). The results of the current study do not provide evidence for a strong influence of the shared environment on verbal abilities in middle childhood.

Genetic and environmental covariation between different verbal tests

Genetic influences appeared to be the driving force behind the covariation between verbal abilities. The genetic effects on the overlap between VIQ and the more specialised verbal tasks were not significantly different from unity in the adolescent cohort, and approached unity in the child cohort (although some of the confidence intervals varied widely). The genetic effects on the overlap between verbal learning, memory, and fluency was modest to strong, and especially strong between letter fluency and verbal learning and memory in both cohorts. The finding of strong genetic influences on the covariance between verbal tests is in line with earlier studies, suggesting that overlap between tests is mainly accounted for by genetic effects, while nonshared environmental effects induce differences in test performance (Deary et

al., 2006; Petrill, 1997; Plomin & Spinath, 2002). However, fitting a model in which the nonshared environmental effects were constrained to be test-specific did not fit the data, neither in the child, nor in the adolescent cohort. This finding implies that these environmental effects, albeit of moderate impact, were also of importance in explaining the overlap between verbal abilities. Possible nonshared environmental effects on verbal abilities could include traumatic experiences unshared with the other family members, or consequences of an accident or illness. Also, if the children are in separate classes, the influences of the teacher or other school-related influences will be nonshared. Furthermore, subject specific influences, such as weariness on the day of testing, may account for the covariance between tests.

Verbal IQ, verbal learning and verbal memory appear to be largely influenced by the same set of genes in late adolescence. The genetic correlations between these measures were close to unity. The genetic correlation between verbal learning and verbal memory in the child cohort was also very high, but its associations with VIQ were somewhat lower. On the whole, the genetic correlations between the different verbal tasks were higher in the adolescent cohort (average r_g=.61) compared to the child cohort (average r_g=.47). This finding is in line with previous studies into specific cognitive abilities, that found a genetic correlation between verbal and nonverbal abilities of about .30 in infancy (Price et al., 2000), increasing genetic correlations between these abilities from early childhood to young adulthood (Hoekstra et al., 2007), up to a genetic correlation of around .70 in adulthood (Hoekstra et al., 2007; Posthuma et al., 2001). The steady increase in genetic correlations between middle childhood and late adolescence in our study suggests a progressive unidimensionality underlying verbal abilities at the genetic level. The high genetic correlations between cognitive domains suggest the existence of "generalist genes": genes that exert a general effect within and between cognitive abilities (Kovas & Plomin, 2006; Plomin & Kovas, 2005). However, a genetic common factor model could not be fit to our data without a significant reduction of the model fit, neither in the child nor in the adolescent cohort, indicating that verbal abilities are not entirely unidimensional. This is also supported by the notion the genetic correlations between verbal learning, memory, and letter and category fluency are still significantly different from one, even in the sample of 18-year-old twins and their siblings.

Implications for future studies

The results from this study are also relevant to research in psychopathology. Several clinical studies have reported impaired performance on verbal learning and memory as measured with the CVLT or the AVLT in patients suffering from schizophrenia (Appels et al., 2003; Egan et al., 2001; Simon et al., 2007; Weickert et al., 2000), or their relatives (Appels et al., 2003; Egan et al., 2001; Snitz et al., 2006; Szoke et al., 2005). Moreover, some studies reported impaired performance on letter or category verbal fluency in schizophrenia patients and their relatives (Appels et al., 2003; Chen et al., 2000; Snitz et al., 2006; Szoke et al., 2005) and in (relatives of) children diagnosed with an autism spectrum disorder (Geurts et al., 2004; Hughes et al., 1999). Following this, measures of verbal learning, memory, and fluency have been proposed as promising endophenotypes for psychiatric illness. One of the criteria for a good endophenotype is that the endophenotype itself should be under substantial genetic control (De Geus & Boomsma, 2001; Viding & Blakemore, 2007). The current study provides a direct test of this criterion. The results of our study indicate that individual differences in verbal learning and memory, as measured with the AVLT, are more strongly genetically determined than verbal learning and memory as measured with the CVLT. Therefore, we suggest the use of the AVLT if researchers plan to use verbal learning and memory as an endophenotype. Both letter fluency and category fluency are under moderate genetic influence, and could therefore both serve as useful endophenotypes. However, it is important to note that the genetic correlation between these measures is relatively low (and not significantly different from zero in the child cohort). Researchers should probably avoid including a composite score of "overall verbal fluency" as an endophenotype, as these measures reflect genetically different cognitive constructs. Lastly, VIQ was under stronger genetic influence than the more specialised verbal abilities in both age groups. The Wechsler VIQ scale comprises an extensive and well validated test battery. Researchers interested in the genetic effects on general verbal abilities should be aware that these abilities are not simply captured by a quick and easy to administer test such as verbal fluency.

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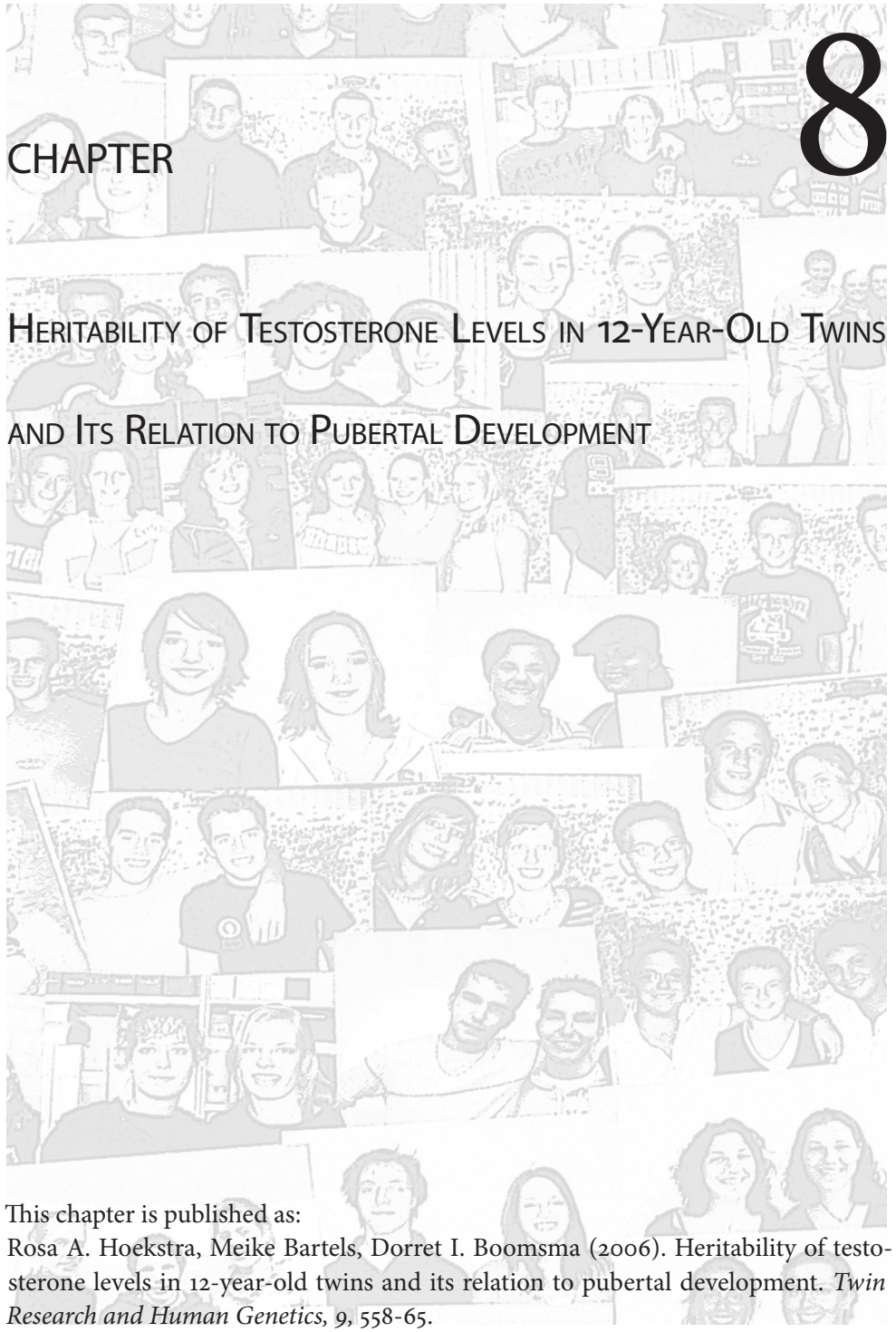

CHAPTER 8

Heritability of Testosterone Levels in 12-Year-Old Twins and Its Relation to Pubertal Development

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Abstract

The aim of this study was to estimate the heritability of variation in testosterone levels in 12-year-old children, and to explore the overlap in genetic and environmental influences on circulating testosterone levels and androgen dependent pubertal development. Midday salivary testosterone samples were collected on two consecutive days in a sample of 183 unselected twin pairs. Androgen induced pubertal development was assessed using self report Tanner scales of pubic hair development (boys and girls) and genital development (boys). A significant contribution of genetic effects to the variance in testosterone levels was found. Heritability was approximately 50% in both boys and girls. The remaining proportion of the variance in testosterone levels could be explained by nonshared environmental influences. The relatively high correlation between testosterone levels of opposite sex dizygotic twins suggests that sex differences in genes influencing variation in testosterone levels have not yet developed in pre- and early puberty. Variance in pubertal development was explained by a large genetic component, moderate shared environmental influences, and a small nonshared environmental effect. Testosterone levels correlated moderately (r=.31) with pubertal development; the covariance between testosterone levels and pubertal development was entirely accounted for by genetic influences.

Introduction

Puberty is the hallmark period in which a child undergoes the transition to adulthood. Driven by hormonal changes, major physical changes occur during this period of development. Puberty starts with the activation of the hypothalamic – pituitary-gonadal (HPG) axis by an increase of the pulsatile release of gonadotropin releasing hormone (GnRH) in the hypothalamus (Grumbach & Styne, 2003; Sisk & Foster, 2004). GnRH induces secretion of luteinising hormone and follicle stimulating hormone in the pituitary, that in turn direct the testes and ovaries to produce sperm and eggs, and activate secretion of steroid hormones. Typically a few years prior to these hormonal changes, children experience the adrenarche, a process biologically independent from the activation of the HPG-axis. Adrenarche results from the activation of the hypothalamic-pituitary-adrenal axis, leading to secretion of dehydroepiandrosterone (DHEA), DHEA-sulphate and androstenedione by the adrenal glands (Auchus & Rainey, 2004; Grumbach & Styne, 2003). Extraglandular conversion of androstenedione leads to increasing levels of the androgens testosterone and dihydrotestosterone.

The increasing levels of circulating hormones lead to different physical changes in puberty. In girls, breast development is induced by increasing estrogen levels, excreted by the ovaries. The development of pubic hair is mainly influenced by androgens, originating from both the ovaries and the adrenal gland. In boys, both genital and pubic hair development are under androgen control (Grumbach & Styne, 2003). Most of the circulating testosterone levels in boys are secreted by the testes. In addition to direct secretion, a small amount of testosterone is derived from peripheral conversion of androstenedione secreted by the testes and adrenal glands (Grumbach & Styne, 2003).

Variation in pubertal timing and individual differences in circulating sex hormone levels are of considerable interest to both the fields of medicine and psychology. Early maturation in girls is found to be associated with internalising symptoms and disorders, and increased rates of alcohol, tobacco and substance use (Graber et al., 2004; Grumbach & Styne, 2003; Hayward & Sanborn, 2002). In boys, late maturation may be a risk factor for deviant behaviour and substance abuse (Graber et al., 2004). Furthermore, individual differences in circulating sex hormone levels are thought to play a role in behavioural problems. An extensive line of research suggests that high testosterone concentrations are linked to (problem) behaviour, particularly aggression (Archer, 1991; Archer, 2006; Book et al., 2001) and dominance (Archer, 2006; Mazur & Booth, 1998). The causality of this relationship is unknown. High testosterone levels could increase the risk of behavioural problems, but behaviour itself can also influence circulating testosterone levels (Archer, 2006; Raine, 2002).

Twin studies show that timing of puberty is influenced by both genetic and environmental effects, with heritability estimates ranging from 50 to 80% (Eaves et al., 2004; Mustanski et al., 2004; Palmert & Boepple, 2001; Van den Berg et al., 2006). Molecular and animal studies suggest polygenic effects. Recent studies indicated that the kiss 1 gene, encoding for a family of neuropeptides called kisspeptines, could play a role in the regulation of GnRH (Popa et al., 2005). Also, the Oct-2 POU domain gene is reported to be associated with the onset of puberty (Terasawa & Fernandez, 2001). Furthermore, nutrition, socioeconomic status (Grumbach & Styne, 2003), stress (Rieder & Coupey, 1999; Wierson et al., 1993) and exercise (Rogol et al., 2000) have shown to affect pubertal timing.

The enzymatic steps controlling testosterone biosynthesis and metabolism have been well characterised (see e.g. Griffin & Wilson, 2003), but the magnitude to which genetic or environmental effects contribute to the variation in circulating testosterone levels is not well established. Studies of heritability of testosterone have focused on adolescent and adult males (Harris et al., 1998; Meikle et al., 1986; Meikle et al., 1988; Ring et al., 2005; Sluyter et al., 2000). Heritability estimates ranged from 26% (Meikle et al., 1986) to 66% (Harris et al., 1998) in males, with the ages of the studied population varying from 14- to 21-year-olds (Harris et al., 1998) to elderly men (Ring et al., 2005). The only study examining heritability in females, found that 41% of the variance in testosterone levels in 14- to 21-year-old women and their mothers was explained by genetic factors (Harris et al., 1998). No studies into the heritability of testosterone levels in children have been reported.

The aim of our current study is to examine heritability of testosterone in 12-year-old children. At this age, variation in testosterone levels will be closely related to the variation in pubertal development. We examined the heritability of both circulating testosterone levels and the pubertal development that is thought to be under androgenic control. Furthermore, the overlap in genetic and environmental influences on testosterone levels and pubertal development is explored.

Methods

Subjects

This project is part of an ongoing longitudinal study on the development of cognition and behavioural problems in children. Subjects were recruited via the Netherlands Twin Register (NTR), kept by the Department of Biological Psychology at the Vrije Universiteit in Amsterdam (Boomsma et al., 1992; Boomsma et al., 2002). Out of the 209 twin pairs initially recruited in 1992, 183 twin pairs participated at age 12 (mean age=12.02; SD=.08; for details see Bartels et al., 2002). Zygosity of the same

sex twins was established by blood group (126 pairs) or DNA polymorphisms (19 pairs) and in four pairs by physical resemblance. The sample consisted of 34 monozygotic male (MZM), 39 dizygotic male (DZM), 40 monozygotic female (MZF), 36 dizygotic female (DZF), and 34 dizygotic pairs of opposite sex (DZMF). Because of difficulties during saliva collection (saliva volume too limited for analysis, or sample contaminated with blood), or laboratory analyses, testosterone samples of 53 children could not be used, resulting in a total sample size of 313 children. Data on androgen dependent pubertal development were available for 172 girls and 165 boys. Since data analyses were performed on the raw data, all available data points were used.

Salivary testosterone collection

Saliva collection devices were sent to the twins by mail and samples were collected at home. Subjects were asked to collect their saliva by passive drool just before lunch, on two consecutive days, since reliability of salivary testosterone measurement is increased by using multiple samples (Dabbs, Jr., 1990). Twins from each pair collected their samples on the same days, and were instructed to do so on two school days to restrict their awakening time and time of sampling. Each participant was asked to write down the exact sampling time in a "saliva diary", and to note exceptional events interfering with the daily routine. Subjects were instructed not to brush their teeth and not to eat or drink in the 30 minutes preceding the saliva collection and to thoroughly rinse their mouth with tap water before sampling. Saliva samples were stored in a refrigerator at the subject's home until completion of the experimental protocol and were collected and brought to the laboratory by the research assistant. Samples were stored at -20°C until radioimmunoassay.

Laboratory analysis

The saliva samples of twins from the same pair were randomly distributed over different batches; the samples of the same subject were analysed together in one batch. By doing so, any laboratory error would be reflected in variance unique to an individual, and not in variance common to a twin pair. All analyses were performed without knowledge of the zygosity of the twins. Testosterone level in saliva samples was measured after diethylether extraction using an in-house competitive radioimmunoassay employing a polyclonal antitestosterone-antibody (Dr.Pratt AZG 3290). [1,2,6,7-3H]-Testosterone (TRK402, Amersham Nederland B.V.) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 10 pmol/L and inter-assay variation was 12.9, 8.3, and 10.6% at 60, 185 and 490 pmol/L respectively (n=24).

Pubertal development

Pubertal status was determined using the self report version of the Tanner scales (Marshall & Tanner, 1969; Marshall & Tanner, 1970). The scales consist of schematic drawings of different pubertal stages of breast (girls), genital (boys) and pubic hair (both boys and girls) development. The scales range from 1 (pre-pubertal) to 5 (post-pubertal) for the assessment of breast and pubic hair development in girls. The pubic hair stages in boys ranged from 1 to 4, the genital development stages in boys ranged from 1 to 5. Additionally, girls were asked whether they had had their menarche yet. In this study, we focused on pubertal development that is known to be influenced by androgen levels. For girls, the pubic hair development score was used. For boys, the mean of the two developmental stages (genital development and pubic hair development) was taken and rounded off to the closest integer. If only one of the male Tanner stages was filled out (n=5), the Tanner stage of this single measure was used as indicator of pubertal development. Studies into the reliability of self report measures of pubertal development have shown that the agreement between ratings of health professionals and self ratings decreases in later stages of puberty. Collapsing the Tanner stages of late puberty into one stage would increase the agreement (Coleman & Coleman, 2002). This finding, together with the fact that very few children reported late stages of puberty in our sample (see results section), made us decide to collapse Tanner stage 3, 4, and 5 into one stage.

Data analysis

Descriptive statistics were calculated using SPSS/Windows 11.5. Testosterone measures were treated as continuous variables, pubertal development was treated as an ordinal variable with three categories. Because the testosterone variables were non-normally distributed, these variables were log transformed before statistical analysis. Analyses of variance were performed to examine testosterone level differences between girls who had had their first menstruation, and girls who had not had their menarche yet. Effects of day of sampling (day 1 vs. day 2), birth order (first born vs. second born), zygosity (MZ vs. DZ) and sex (males vs. females) on testosterone level means were examined using a bivariate saturated model in the computer programme Mx (Neale et al., 2003). Phenotypic correlations between the two testosterone measures were estimated in Mx, polyserial correlations between testosterone and pubertal development were estimated in Mplus (Muthén & Muthén, 2006).

Genetic modelling

In order to estimate the proportion of the variance arising from additive (A) genetic effects, dominance effects (D), shared (C) and nonshared (E) environmental influences, genetic modelling was performed in two steps, using both Mx (Neale et al., 2003) and Mplus (Muthén & Muthén, 2006). To get an impression of which variance components are of importance, first a saturated bivariate model for testosterone levels on the two measurement occasions was fitted using Mx. Based on the twin correlations, an ADE model was applied and evaluated using the -2 log likelihood (-2LL). The significance of sex differences on the magnitude of A, D, and E and the significance of variance components A and D was assessed by testing the deterioration in model fit after each component was dropped from the full model. The deterioration of the model was evaluated using the likelihood ratio test, the difference between the -2LL under the two nested models, which is asymptotically distributed as a χ^2. The degrees of freedom are given by the difference in the number of parameters estimated in the two models. A high increase in χ^2 against a low gain of degrees of freedom denotes a worse fit of the submodel compared to the full model. The most parsimonious model, with still a limited χ^2 was chosen as the best model.

Secondly, a trivariate model consisting of two continuous variables (testosterone levels on two measurement occasions) and one ordinal variable (pubertal development) was fitted in Mplus (Muthén & Muthén, 2006; see Prescott, 2004 for specifying twin models in Mplus). A liability model with thresholds was fitted for pubertal development, assuming that pubertal development is a gradual process and subjects who exceed an underlying threshold are in a more advanced stage of puberty than subjects who score below this threshold. All models were fitted allowing parameter estimates for means and thresholds to differ across males and females. Initially, sex differences in magnitude of the variance components were also allowed. A series of models were fitted to the data, using weighted least squares with mean and variance adjusted chi-squares (WLSMV). When comparing nested models using WLSMV in Mplus, the χ^2 and degrees of freedom are adjusted (see the technical appendix of Mplus or the Mplus discussion board, both on www.statmodel.com). The only value that is interpretable is the p-value. Therefore, only the p-values are mentioned in the results section.

To test whether different genes affect pubertal development in males and females, we freely estimated the correlation between the genetic effects for opposite sex DZ twins. An estimated correlation significantly lower than 0.5 would indicate that opposite sex DZ twins share less genetic variance than same sex DZ twins. The estimate of the correlation was constrained to fall within the range of biologically plausible values (0 - 0.5) first, and consequently constrained to the value of 0.5. Adjusted chi-squared difference testing using WLSMV is not available in Mplus 4.0 in combination with boundary constraints. Therefore weighted least squares with mean adjusted chi-squares (WLSM) was used in this case, for which a simplified version of the adjusted chi-squared difference test was presented in Satorra & Bentler (1999; see also the technical appendix of Mplus, page 22, on www.statmodel.com). We estimated

the simplified chi-squared difference to test if the estimated opposite sex DZ correlation differed significantly from 0.5. Similarly, we tested whether the correlation of shared environmental effects on pubertal development was lower in opposite sex twins than in same sex twins. An estimated correlation lower than 1.0 would indicate that opposite sex twins have less shared environmental influences in common than same sex twins. The estimate of the correlation was bound to fall between 0 and 1. Subsequently it was tested whether the shared environmental correlation in opposite sex twins was significantly different from zero.

Results

Table 1 shows the scores on the Tanner scales in boys and girls. The majority of the children reported to be in the early to mid stages of puberty: 81.9% (genital development) and 98.2% (pubic hair growth) of the boys reported to be in early to mid-pubertal stages. Of the girls, respectively 88.5% and 78.5 % reported to be in early to mid stages of breast development and pubic hair growth. Twenty girls (both from the MZ and DZ twin groups) reported that they had experienced their first menstruation. Testosterone levels in these girls were significantly increased compared to those who had not yet had their first menstruation, $F(1, 149)=6.144$, $p=.014$. No outliers were

TABLE 1. Frequency distributions of pubertal Tanner stages for breast development and pubic hair growth in girls and genital development and pubic hair growth in boys.

Tanner stage	♀ (n=174) Breast development	♀ (n=172) pubic hair growth	♂ (n=160)* genital development	♂ (n=163)* pubic hair growth
1	38 (21.8%)	47 (27.3%)	24 (15%)	85 (52.1%)
2	66 (37.9%)	41 (23.9%)	27 (16.9%)	71 (43.6%)
3	50 (28.8%)	47 (27.3%)	80 (50%)	4 (2.5%)
4	20 (11.5%)	33 (19.2%)	27 (16.9%)	3 (1.8%)
5	0	4 (2.3%)	2 (1.2%)	N/A

* Number of boys for which information on either one of the pubertal stages is available is 165.

detected; all testosterone levels fell within a range of 2 SDs from the mean. Therefore it was decided to retain all data in the analysis. In subsequent analyses using the androgen dependent Tanner scales, stage 3 and above were collapsed into one stage.

Descriptive statistics on the testosterone measures for the two samples are presented in Table 2. Sample size, mean testosterone levels and standard deviations are shown for both boys and girls. Moreover, testosterone levels are shown per stage of androgen dependent pubertal development. Testosterone levels were significantly correlated with pubertal development: r=.31 (95% CI=.20 - .43), this correlation was equal for both testosterone measurements, and equal in boys and girls. Within person correlation for the testosterone samples taken on the two consecutive days was .55 (95% CI=.46 - .64) There were no birth order effects (differences in testosterone

TABLE 2. Descriptive statistics for testosterone levels (pmol/L) in sample 1 (s1) and sample 2 (s2) in all boys (♂) and girls (♀), and separate for each stage of pubertal development.

	N	Mean		N	Mean
Total					
♂s1	140*	71.6 (23.2)	♀ s1	145*	85.4 (29.9)
♂s2	134*	70.7 (24.2)	♀ s2	145*	81.3 (28.1)
Per stage of pubertal development					
♂s1 Stage 1	14	69.9 (21.7)	♀s1 Stage 1	32	71.4 (24.6)
♂s2 Stage 1	17	68.2 (23.4)	♀s2 Stage 1	35	70.6 (21.8)
♂s1 Stage 2	57	63.9 (18.9)	♀s1 Stage 2	35	77.3 (21.7)
♂s2 Stage 2	54	67.2 (16.1)	♀s2 Stage 2	38	81.5 (32.4)
♂s1 Stage ≥ 3	58	78.7 (25.5)	♀s1 Stage ≥ 3	71	94.8 (32.5)
♂s2 Stage ≥ 3	52	73.9 (27.3)	♀s2 Stage ≥ 3	63	86.9 (26.9)

* Tanner stage information missing for 11 boys in both sample 1 and 2. Tanner stage information missing for 7 girls in sample 1 and for 9 girls in sample 2.

TABLE 3. Twin correlations and cross-correlations per zygosity for testosterone sample 1 and 2 (estimated in Mx using maximum likelihood) and for pubertal development (polychoric correlations in Mplus). 95% confidence interval between parentheses.

	Testosterone sample 1	Testosterone sample 2	Twin cross $r_{s1\text{-}s2}$	Pubertal development	Twin cross $r_{s1s2\text{-}pd}$
MZM	.76 (.55-.88)	.67 (.41-.83)	.49 (.22-.69)	.97 (.93-1.0)	.03 (-.34-.39)
DZM	.12 (.00-.46)	.00 (.00-.21)	.02 (-.12-.24)	.75 (.52-.99)	.25 (.02-.48)
MZF	.51 (.17-.73)	.58 (.30-.77)	.35 (.07-.58)	.96 (.89-1.0)	.21 (-.04-.45)
DZF	.27 (.00-.57)	.23 (.00-.53)	.25 (-.03-.52)	.73 (.48-.97)	.28 (.05-.51)
DZMF	.23 (.01-.55)	.53 (.16-.77)	.35 (.06-.60)	.15 (-.31-.60)	.15 (-.11-.41)

MZM = monozygotic male twin pairs; DZM = dizygotic male twin pairs; MZF = monozygotic female twin pairs; DZF = dizygotic female twin pairs; DZMF = opposite sex twin pairs. s1 = testosterone sample 1; s2 = testosterone sample 2; pd = pubertal development.

level between first- and second-borns, χ^2=3.028, df=4, p=.553) or zygosity effects (mean testosterone level DZ vs. DZMF twins, χ^2=1.465, df=2, p=.481; MZ vs. DZ twins, $\chi^{2=}$0.545, df=2, p=.761). Testosterone levels were higher in girls than in boys ($\chi^{2=}$20.773, df=1, p<.001).

Twin correlations for testosterone levels (see Table 3) showed that MZ correlations were higher than DZ correlations in both sexes, indicating additive genetic effects (A). In boys, the DZ correlation for testosterone levels was less than half the MZ correlation, suggesting dominance effects. The relatively high correlation in DZ opposite sex twins (r=.23 for sample 1 and r=.53 for sample 2) suggests overlap in genetic expression in boys and girls. A bivariate ADE model of testosterone levels on two measurement occasions was fitted. Constraining the parameters that represent the influence of A, D and E to be the same in boys and girls did not significantly worsen the fit ($\chi^{2=}$8.373, df=9, p=.497), suggesting that the relative effects of these components were equal in both sexes. Dropping component D from the model did not result in a worse model fit ($\chi^{2=}$1.661, df=3, p=.646). The best fitting parsimonious model was a model in which variation of testosterone levels was accounted for by additive genetic influences and nonshared environmental effects.

Twin correlations for androgen dependent pubertal development (see Table 3) showed very high MZ correlations and rather high DZ correlations, suggesting both genetic and shared environmental effects. The point estimate for the correlation of opposite sex DZ twins was low, but the confidence interval for this correlation was rather wide. A trivariate model incorporating both testosterone measures and pubertal development was fitted in Mplus. Based on the outcome of the analyses in Mx, an AE model was tested for the testosterone variables. Based on the twin correlations, the variance unique to pubertal development (i.e. the variance that could not be explained by additive genetic or nonshared environmental variance in testosterone) was partitioned into components A, C and E. Genetic model fitting showed that the genetic correlation of pubertal development in opposite sex DZ twins could be equalled to 0.5, similar to same sex DZ twins (χ^2=.069, df=1, p=.792). Constraining the parameters that represent the influence of A and E on testosterone levels to be the same in boys and girls did not significantly worsen the fit (p=.823), indicating that the relative effects of these components are equal in both sexes. Similarly, constraining the variance components A, C and E on the residual variance of pubertal development to be equal in both sexes, did not significantly worsen the fit (p=.221). The shared environmental influences on the variance unique to pubertal development were of significant importance, dropping the C component from the model lead to a significant deterioration of the model (p=.024). Constraining the influence of E on the covariance between the variables to be zero did not significantly affect the fit of the model (p=.304), indicating that the nonshared environmental influences were unique for all measurements. The genetic influences on both measurement occasions of testosterone could be constrained to be from the same source (p=.265), indicating that all genetic influences on the testosterone measurements were shared between the two samples. The genetic effects on the covariance between testosterone and pubertal development were of significant importance, dropping this component from the model resulted in a significant deterioration of the fit (p<.001). Also, the genetic influences on the variance unique to pubertal development were significant (p=.016). The best fitting model incorporating both testosterone measures and androgen dependent pubertal development is given in Figure 1.

Heritability estimates were calculated by squaring the standardised estimates of the path loadings. In the best fitting model, additive genetic effects explained $(.720)^2$=52% of the variance in testosterone levels on both measurement days. The nonshared environmental influences on T-levels were unique to both measurement occasions, and could explain $(.694)^2$=48% of the variance. Fifteen percent of the variance ($(.391)^2$) in pubertal development was shared with variance in testosterone levels, and this covariance was entirely accounted for by genetic effects. The variance unique to pubertal development was accounted for by genetic effects (45%); shared

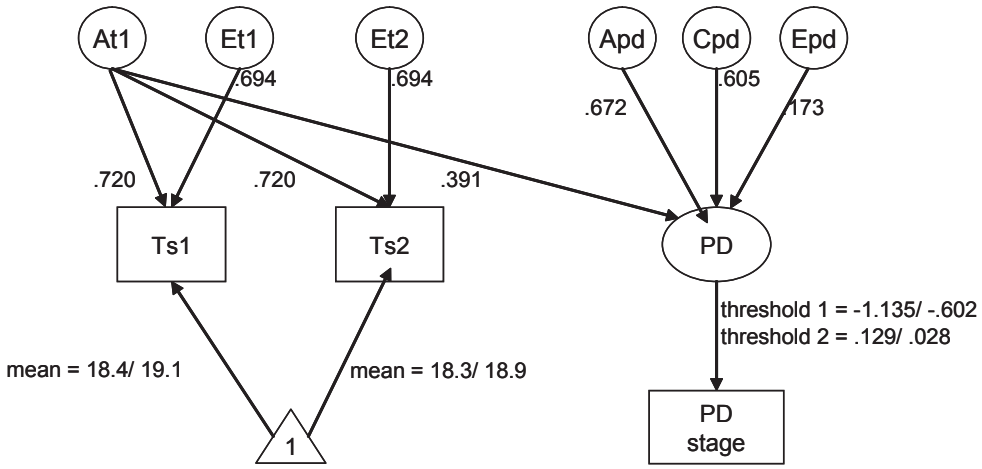

FIGURE 1. Path diagram of the best fitting model with standardised estimates for the different variance components. Observed variables are shown in boxes; latent variables are depicted in circles and ovals; the model for the means is represented by the triangle. Ts1 = testosterone level in sample 1; Ts2 = testosterone level sample 2; PD = Underlying pubertal development, as measured with the ordinal variable pubertal developmental stage. Means and thresholds are estimated for boys (first value) and girls (latter value) separately. At1 = genetic influence on individual differences in testosterone levels; Et1/2 = contribution of nonshared environmental influences on variance in testosterone levels in sample 1 (Et1) and sample 2 (Et2). Apd/Cpd/Epd = Influence of additive genetic (Apd), shared environmental (Cpd) and nonshared environmental (Epd) factors on individual differences unique to pubertal development.

environmental effects (37%) and a small nonshared environmental effect (3%). Lastly, it was tested whether the correlation of shared environmental influences might be lower in opposite sex twins. The shared environmental correlation in opposite sex twins was not significantly different from zero (χ^2=0.037, df=1, p=.847), suggesting that opposite sex twins may have less environmental variance in common than same sex twins.

Discussion

This is the first study to report heritability estimates of testosterone levels in early adolescence. A significant contribution of additive genetic effects to midday salivary testosterone levels was found. The relative contribution of genetic and environmental influences on testosterone level variation was the same in both sexes. Genetic influences explained 52% of the variation. The remaining proportion of the variance

was accounted for by nonshared environmental influences. Moreover, a significant overlap in genetic influences was found between testosterone levels and androgen dependent pubertal development. Fifteen percent of the variance in pubertal development was shared with the variance in testosterone levels. The overlap was entirely explained by genetic effects.

Previous studies have shown that environmental influences, such as participating in sports competitions (and especially winning a competition), could lead to changes in testosterone levels (Archer, 2006). Apart from such 'real' environmental influences, nonshared environmental influences also include measurement error. In this respect it is important to note that both the MZ correlation for testosterone levels (r ranging from .51 to .76) and the MZ cross twin cross sample correlation (cross r=.35 - .49) were as high as the within person correlation for both testosterone samples (r=.55). This finding suggests that, if measurements were corrected for daily fluctuations and measurement error, variance in testosterone levels would practically be entirely explained by genetic effects. This is also reflected in the observation that all covariance between the two testosterone samples is of genetic origin, and all unique variance is nonshared environmental.

Harris et al. (1998) found a heritability estimate of 66% in 14- to 21-year-old males, and an estimate of 41% in females (14- to 21-year-old twins and their mothers). Furthermore, they found zero correlation between testosterone levels of opposite sex DZ twins. In our study, neither sex-specific genes nor sex differences in heritability estimates were detected. The opposite sex DZ twin correlation for testosterone level was .23 and .53 in our sample, suggesting an overlap in genetic expression in boys and girls. Our subjects were younger than the participants in Harris' study, and were still in the early to mid-stages of puberty. Concentrations of circulating testosterone levels are subject to large changes over the lifetime, particularly during puberty. After a surge of testosterone levels in male foetuses, levels are equally low in boys and girls after birth, and remain suppressed until the reactivation of the GnRH neurons, marking the onset of puberty (Grumbach & Styne, 2003). During pubertal development, sex differences in testosterone levels arise, from then onwards testosterone levels are higher in males than in females (Grumbach & Styne, 2003). In our sample, testosterone levels were found to be significantly higher in girls than in boys, indicating that the majority of the boys was still prepubescent. A study in adolescent twins and their parents found no correlations in plasma testosterone levels between fathers and sons, suggesting that different genetic mechanisms may influence testosterone concentrations during the life span (Harris et al., 1998). The results of our study suggest that in pre- and early puberty, there are no sex differences in genes influencing variation in testosterone levels. Neither differences in the magnitude of the variance components, nor significant influences of dominance effects or shared

environmental factors were found on the variation in testosterone levels. However, our sample size was relatively small and a contribution of shared environmental or genetic dominance effects, or subtle differences in the magnitude of A and E on the variance in testosterone levels cannot completely be excluded, due to statistical power considerations.

Variance in androgen dependent pubertal development was largely (60%) explained by genetic effects. Shared environmental effects could explain 37% of the variation in pubertal development. Nutrition and other factors related to socio-economic status have been shown to influence pubertal development (Grumbach & Styne, 2003), these factors could explain the non-genetic familial clustering. The twins completed the puberty questionnaire at home. Therefore, it cannot be ruled out that the twin resemblance was inflated because twins discussed their ratings. This explanation is further supported by the finding that the shared environmental correlation in opposite sex twins (who could not discuss their ratings because they filled out different questionnaires) did not significantly differ from zero, suggesting less shared environmental variance in pubertal development in opposite sex twins than in same sex twins.

In summary, this study provides evidence that the variation in salivary testosterone levels in 12-year-old children is explained by genetic and nonshared environmental effects in both boys and girls. The relatively high correlation between testosterone levels in opposite sex DZ twins suggests that sex differences in gene expression arise in more advanced stages of puberty. At this age, fifteen percent of the variance in androgen induced pubertal development is shared with the variance in testosterone levels. This overlap is entirely accounted for by genetic influences.

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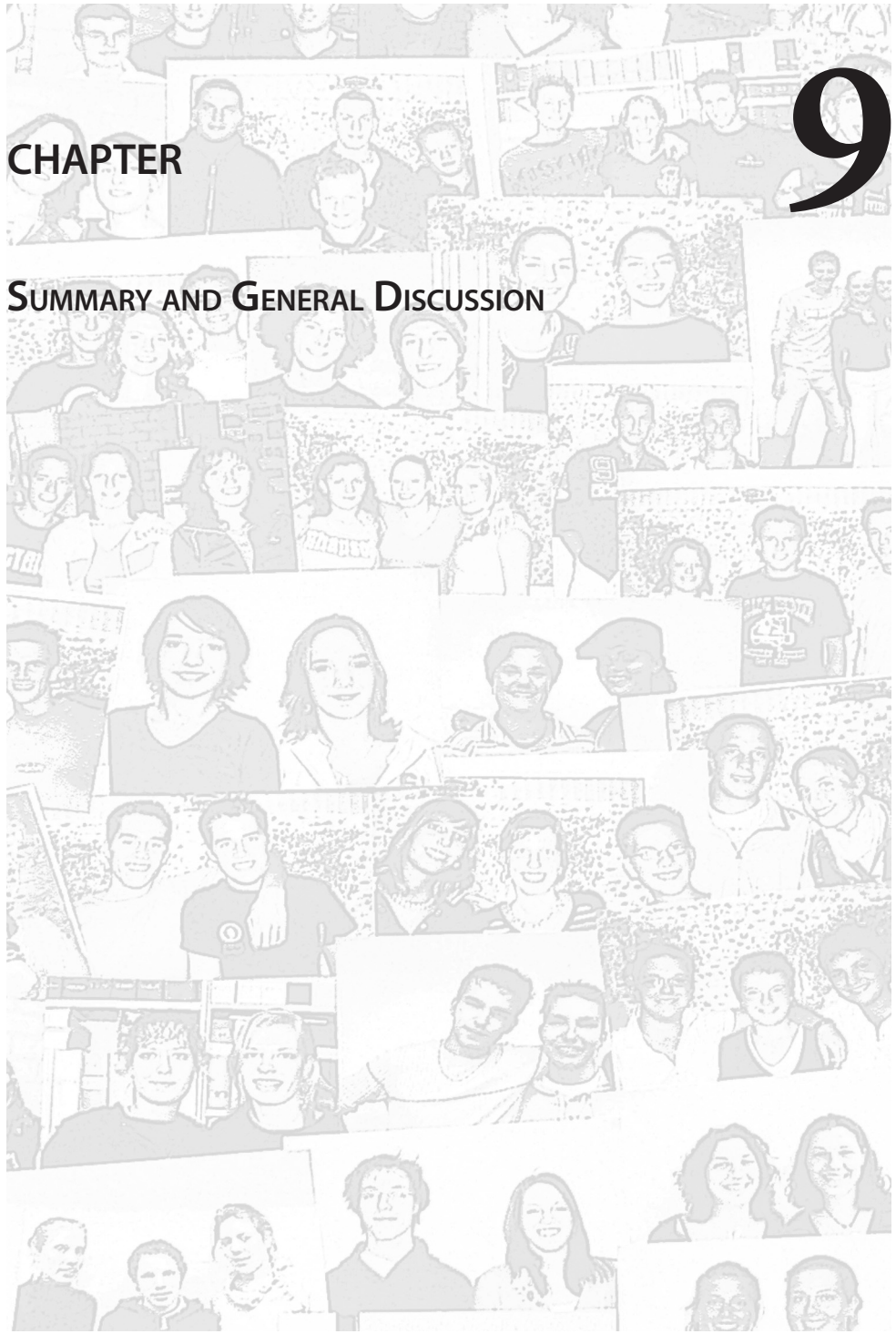

CHAPTER 9

Summary and General Discussion

This thesis describes the study of individual differences in autistic traits and withdrawn behaviour and the development of cognitive abilities. Data on cognition and problem behaviour were collected in a group of 209 twin pairs when they were 5, 7, 10, 12, and 18 years of age. At the last measurement occasion, the siblings of the twins were also invited to participate. Verbal and nonverbal IQ data were collected at all time points. At the fifth measurement occasion, performance on additional verbal ability tasks was assessed and the participants completed a questionnaire measuring endorsement of autistic traits. At age 12 and 18 years, the participants were asked to collect salivary testosterone samples and to fill out a questionnaire assessing pubertal development. In the last chapter of this thesis, the findings that have resulted from this project are summarised and discussed and some directions for future studies are considered.

Variation in autistic traits

Chapters 2 to 4 were devoted to the examination of variance in autistic traits and its association with behavioural problems. Chapter 2 started with determining the reliability and validity of the Dutch translation of the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001b), a self-report measure to assess autistic traits. AQ scores were collected in a large student sample and a general population sample. Test-retest data were available from a sub sample of the participants in the twin family study. Autistic traits were continuously distributed in all samples. The Dutch AQ showed good psychometric properties, with an internal consistency of α=.79 and a test-retest reliability of .78. Men obtained significantly higher AQ scores than women, which is in line with the observation that autism is much more common in men than in women (Fombonne, 2003) and with previous studies using a dimensional approach to assess autistic traits (Baron-Cohen et al., 2001b; Constantino & Todd, 2003; Ronald et al., 2006; Wakabayashi et al., 2006). Science students scored significantly higher than students enrolled in a humanities or social sciences degree. This is in line with the finding that relatives of autistic individuals more often have a profession in the science domain, such as engineering (Baron-Cohen et al., 1997) and mathematics (Baron-Cohen et al., 2007) and with the finding that students with a parent in a scientific occupation tend to score higher on the AQ (Austin, 2005).

The diagnostic validity of the Dutch AQ was examined in three matched patient groups, i.e. subjects diagnosed with an autism spectrum condition (ASC), social anxiety disorder (SAD), or obsessive compulsive disorder (OCD). The ASC patients scored significantly higher than the SAD and OCD patients, indicating that very high scores on the AQ are specific to individuals with an ASC diagnosis. Within the ASC group, patients with (high functioning) autism and Asperger syndrome obtained the highest scores (mean AQ=160.8, sd=13.6). The AQ scores of patients

diagnosed with pervasive developmental disorder-not otherwise specified (mean AQ=123.7, sd=7.2), a broad diagnostic category with criteria less stringent than for autistic disorder (American Psychiatric Association, 2000), fell in between the scores for autism and Asperger syndrome patients, and scores from SAD and OCD patients (mean AQ 114.5; sd=14.4), who in turn scored higher than the general population (mean AQ=104.2, sd=11.3). In concordance with previous studies (Baron-Cohen et al., 2001b; Constantino & Todd, 2003; Piven et al., 1997; Spiker et al., 2002), these results suggest that, rather than a distinct disorder, the clinical diagnosis of autism represents the upper extreme of a constellation of traits that are continuously distributed in the general population. The factor structure of the AQ was also examined in chapter 2, and two underlying factors were identified. One factor encompassed broad problems with social interaction, the other factor focused on a preference and talent for attention to detail. Thus, this chapter indicated that the Dutch translation of the AQ is a reliable instrument to assess endorsement of autistic traits.

Using the instrument that was validated in chapter 2, the genetic and environmental influences on individual differences in autistic traits in 18-year-old twins and their siblings were investigated in chapter 3. Variance in endorsement of autistic traits was under substantial genetic control, 57% of the variance could be attributed to genetic effects. The remaining variance was explained by unique environmental factors, i.e. environmental factors that were not shared by twins and siblings growing up together. The resemblance in opposite sex twins was similar to the resemblance in same sex dizygotic twins, yielding no evidence for sex-specific genetic effects on the variance in autistic traits. Twins did not differ in their mean AQ scores from their non-twin siblings, indicating that endorsement of autistic traits is unrelated to being born a twin or a singleton. Previous general population studies indicated that individual differences in endorsement of autistic traits are moderately to highly heritable in middle childhood and early adolescence (Constantino & Todd, 2000; Constantino & Todd, 2003; Ronald et al., 2005; Ronald et al., 2006). Our twin family study extends these findings and shows that autistic traits are also under substantial genetic control in late adolescence. Lastly, we examined the spousal resemblance for endorsement of autistic traits in a general population sample. The correlation between the AQ scores of spouses was low and not significant, suggesting that there is no active or passive partner selection for autistic traits in the general population. Thus there is no indication that the heritability estimate found in our twin family study could be biased due to assortative mating.

Chapter 4 explored the covariation between autistic traits and behavioural and emotional problems as indexed by Youth Self Report ratings (YSR; Achenbach & Rescorla, 2001; Verhulst et al., 1997). Stepwise multiple regression analyses showed that the YSR syndrome scales Withdrawn Behaviour (WB) and Social Problems

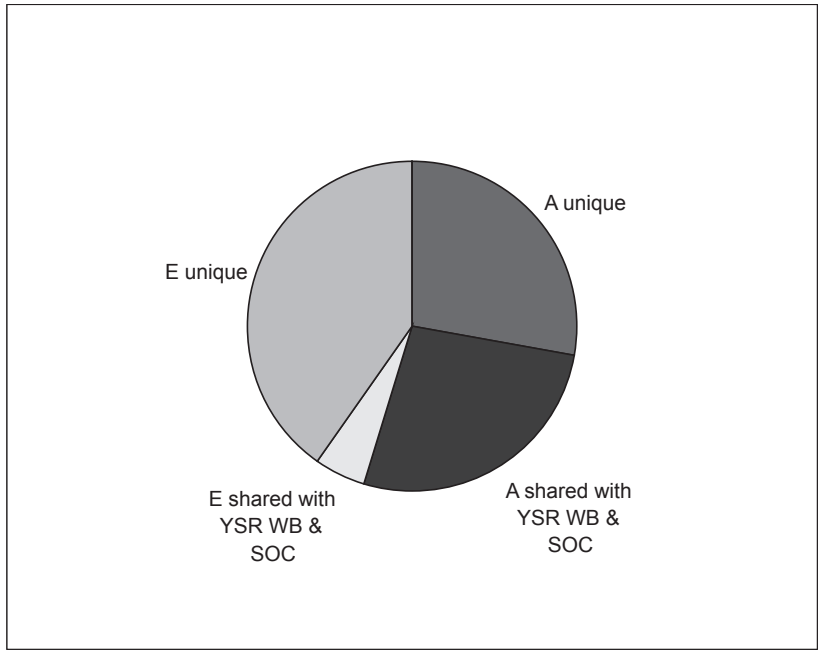

Figure 1. Pie chart representing the different sources of variation in autistic traits.

(SOC) were significant predictors of endorsement of autistic traits. Together with sex, WB and SOC explained 23% of the variance in AQ scores. Subsequent multivariate genetic modelling revealed that the overlap between these YSR scores and the AQ was mainly due to genetic effects. About half of the genetic variance in autistic traits was shared with variance in WB and SOC scores; the remaining genetic variance was specific to the AQ. The greater part of the nonshared environmental variance was specific to the AQ, only 11% of the nonshared environmental variance in AQ scores showed an overlap with the variance in YSR scores. In conclusion, this chapter indicates that individual differences in autistic traits in the general population are associated with social and withdrawn behavioural problems, and that these problems partly share a common genetic aetiology. However, most of the variance in AQ scores remains unexplained by scores on the YSR, indicating that autistic traits are substantially independent from other behavioural problems.

Figure 1 summarises the sources of variation in autistic traits as found in the studies reported on in chapters 2 to 4. Additive genetic influences (A) explain 57% of the variance in autistic traits in late adolescence. About half of the genetic variance in AQ is shared with genetic variance in WB and SOC scores. The remaining variance is accounted for by nonshared environmental influences (E), of which a small proportion is also common to the nonshared environmental variance in YSR scores. The test-

retest reliability of the AQ was .78. This suggests that up to 39% ((1 - $.78^2$)*100%=39%) of the variance in AQ scores is not stable over time and may be due to e.g. measurement error. Variance due to scale unreliability is included in the unique environmental influences, leaving little room for other nonshared environmental effects on the variance in autistic traits. These results indicate that the strong heritability of autistic traits is not limited to the clinical spectrum. Genetic effects also account for a substantial part of the variance in autistic traits in the general population. These findings also have implications for linkage and association studies. Rather than using a discrete measure of autism (affected vs. unaffected), genetic studies may be facilitated by measuring autistic traits on a quantitative scale such as the AQ.

Withdrawn behaviour

Chapter 5 described a longitudinal study of childhood withdrawn behaviour, using both maternal and paternal ratings of the twin's behaviour at ages 3, 7, 10, and 12 years on the Withdrawn Behaviour subscale of the Child Behavior Checklist (Achenbach & Rescorla, 2001; Verhulst et al., 1996). The heritability estimates at different time points in childhood (see Figure 2) show that individual differences in withdrawn behaviour are under substantial genetic influence. Between age 3 and 12 years, genetic effects explain 50 to 66% of the variance in boys and 40 to 61% of the variance in girls. Shared environmental influences explained a modest but significant part of the variance (2-23%) in childhood and were somewhat stronger in girls than in boys. Nonshared environmental influences were moderate (21-41%) at all childhood ages and in both sexes.

Withdrawn behaviour showed considerable stability throughout childhood, with correlation coefficients of .23 to .29 for the stability between ages 3 to 12, up to correlations of .59 to .65 for the stability between age 10 and 12 years. Genetic effects appeared to be the driving force behind the continuity of withdrawn behaviour throughout childhood, and explained 74% of the stability in boys, and 65% in girls. Shared environmental effects explained 8% (boys) and 18% (girls) of the continuity of withdrawn behaviour. Most of these effects (4% in boys; 13% in girls) were common to both raters, suggesting that these are real influences of the shared environment and not due to rater bias. The remaining covariance is accounted for by nonshared environmental influences. About two-third of these effects were common to both raters, indicating that these were true nonshared environmental effects, and not reflections of unreliability. Some studies suggest that parenting style, such as inappropriate affectionate parenting (Park et al., 1997) or maternal over-control (Rubin et al., 2002) may affect the stability of inhibited and shy behaviour in young children. If the parent displays the parenting style to both members of the twin pair, this influence would be represented in the shared environmental component. A child specific style

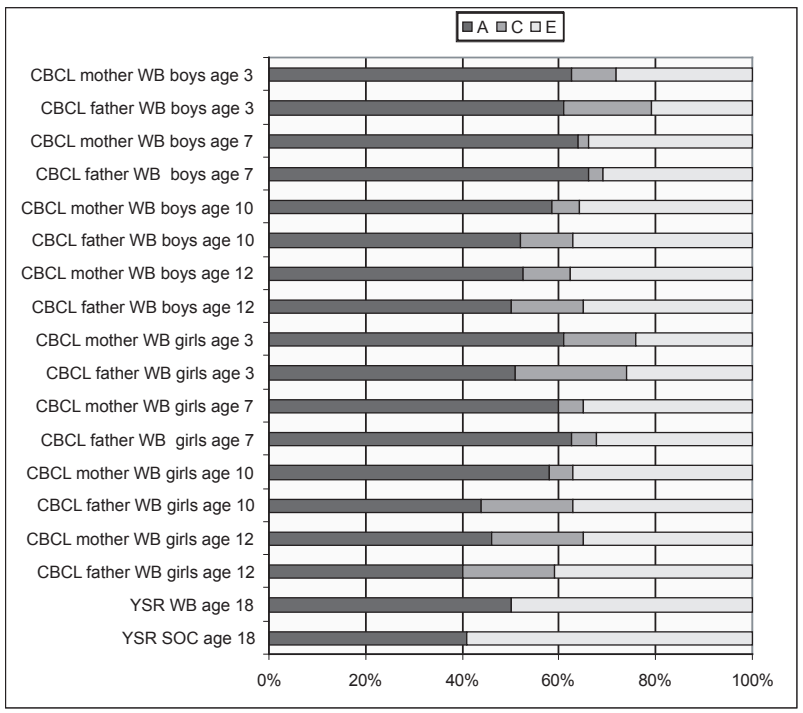

Figure 2. Percentage of the total variance explained by additive genetic (A), shared environmental (C), and nonshared environmental (E) effects in withdrawn behaviour (WB) and social problems (SOC) in 3-, 7-, 10-, 12-, and 18-year-olds, as assessed by maternal and paternal Child Behavior Checklist (CBCL) report and Youth Self Report (YSR).

of parenting would show up in the nonshared environmental component. The genetic and shared environmental correlations across time of the phenotype common to both raters approached unity, suggesting that the set of genes and shared environmental factors that influence withdrawn behaviour remains the same throughout childhood.

The analysis of YSR (Achenbach & Rescorla, 2001; Verhulst et al., 1997) ratings of withdrawn behaviour in 18-year-old twins and their siblings (as reported on in chapter 4) provide a first insight on the factors influencing withdrawn behaviour in late adolescence. The heritability was 50%, indicating that genetic influences remain of importance in explaining individual differences in a later phase of development. At this age, shared environmental effects were insignificant. The number of families for whom YSR data were available (424 families) is lower than the number of families involved in the childhood WB study (14,889 families), and at first sight the lack of shared environmental effects on WB in late adolescence may seem to be due to lack of power (Posthuma & Boomsma, 2000) to detect these influences. However, the

twin correlations for YSR withdrawn behaviour scores at age 18 (rMZ=.55; rDZ=.28) provide little evidence for shared environmental effects at this age. Thus, although the vast majority of the participating twins and siblings still lived with their parents (92%, according to the data from our questionnaire study), environmental influences on variance in withdrawn behaviour at age 18 year appear to be largely nonshared. Throughout adolescence , Western youth spend progressively less time in the family environment, and more time with peers and at school (Larson & Verma, 1999). These influences, if unshared with the other members of the family, would be reflected in the nonshared environmental component.

Chapter 4 also reported on the genetic and environmental influences on self-reported social problems (see also Figure 2). Genetic influences on variance in social problems in 18-year-old twins and their siblings were moderate and explained 41% of the variance. The remaining variance was accounted for by non-shared environmental effects. Apart from behavioural ratings at age 18, the Netherlands Twin register has started collecting YSR data of twins and their siblings when the twins are 14 and 16 years of age. This is an ongoing data collection and the available longitudinal data is still growing. Future studies using these longitudinal data will be able to shed a light on the stability of withdrawn behaviour in adolescence.

Cognitive abilities

The study of cognitive abilities has received ample attention in the field of behaviour genetics. This thesis aimed to contribute to the existing literature by studying verbal and nonverbal intelligence from early childhood to young adulthood, and by examining the genetic architecture underlying covariance in specific verbal abilities in middle childhood and early adulthood. By analysing longitudinal IQ data collected in twins when they were 5, 7, 10, 12, and 18 years of age, the genetic and environmental factors underlying stability in verbal and nonverbal abilities were explored in chapter 6. Both verbal and nonverbal IQ showed high stability, with correlation coefficients over time ranging from .47 for the 13-year time interval, up to .80 for the shorter time intervals. Consistent with previous studies (Bartels et al., 2002; Bishop et al., 2003; Petrill et al., 2004; Posthuma et al., 2002; Wilson, 1983), multivariate longitudinal genetic analyses showed increasing influence of genetic effects with age, from 48% to 84% for verbal IQ, and from 64% to 74% for nonverbal IQ (see Figure 3). The shared environmental component only had a significant influence on the variance in verbal IQ in early and middle childhood. In line with previous studies (Bartels et al., 2002; Bishop et al., 2003), these effects ceased with age and became insignificant when the twins reached adulthood. Genetic effects were the main source for explaining stability in verbal and nonverbal abilities over time. The continuity in nonverbal IQ was entirely accounted for by genetic factors. Stability in verbal IQ was explained

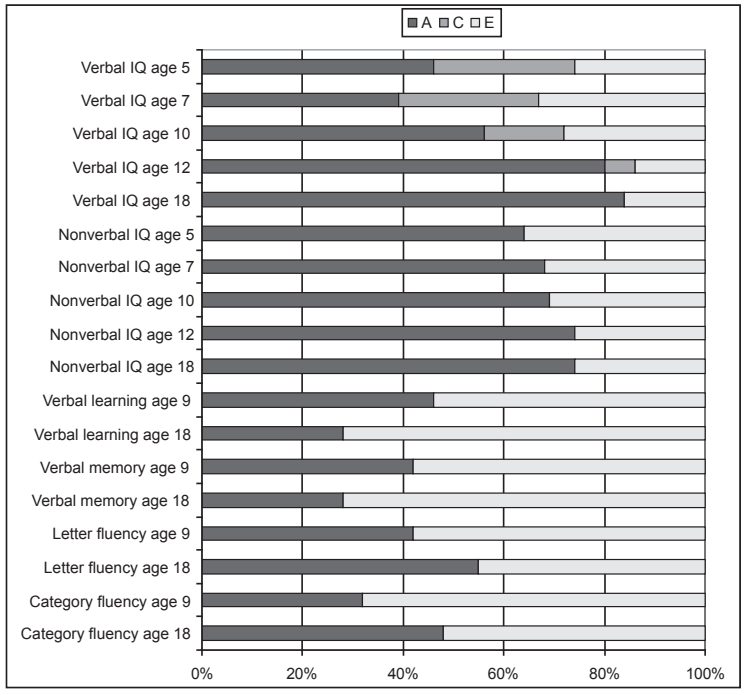

Figure 3. Percentage of the total variance explained by additive genetic (A), shared environmental (C), and nonshared environmental (E) effects in verbal and nonverbal IQ at ages 5, 7, 10, 12 and 18 years, and in verbal abilities at age 9 and 18 years.

by strong genetic influences and moderate shared environmental effects, with the latter only exerting an influence on stability from early to middle childhood. Nonshared environmental influences were only important for explaining age-specific variance and did not contribute to the stability in verbal and nonverbal abilities over time. The covariance between verbal and nonverbal abilities was entirely accounted for by genetic effects. The genetic correlation between these abilities increased slightly over the years, from .62 when the twins were 5 years old to .73 when they had reached the adult age, suggesting that the overlap in the set of genes influencing different cognitive abilities becomes stronger with age.

Chapter 7 described a twin family study of verbal abilities in childhood and adolescence. The aetiology of individual differences in verbal IQ, verbal learning, verbal memory, and letter and category fluency was examined in two independent samples of 9-year-old and 18-year-old twins and their siblings, and the sources of covariation between these abilities were explored. In both samples, the resemblance between dizygotic twins was similar to the resemblance between twins and their non-twin siblings, yielding no evidence for a twin-specific environment for verbal abilities. In

both the child and the adolescent cohort, the heritability of verbal IQ was strong (82 and 84%), while the genetic influences on the other verbal measures were moderate (28-55%; see Figure 3). Shared environmental influences were not significant in either of the cohorts. Against expectation, the genetic effects on verbal memory and verbal learning did not increase with age, when comparing cross-sectional data from 9-year-old twins and their siblings with data from 18-year-old twins and their siblings. Verbal learning and memory performance was assessed using slightly different tests in these two cohorts. In the child cohort, learning and memory of unrelated words was assessed, while the older cohort was asked to memorise words belonging to certain categories. A previous study (Volk et al., 2006) reported stronger heritability for uncategorised word learning than categorised word learning, which may explain the relatively low heritability estimates for learning and memory found in our cohort of 18-year-old twins and their siblings. Genetic factors accounted for most of the covariance between the tests (70 and 74%), the remaining covariance was explained by nonshared environmental influences. The main difference between both cohorts concerned the phenotypic and genetic correlations between the different tests. Both the phenotypic and the genetic overlap between the verbal tests was stronger in the adolescent cohort than in the child cohort, suggesting progressive unidimensionality in verbal abilities with age.

The general picture that can be drawn from the studies described in chapter 6 and 7 is that covariance between tests (either the covariance between nonverbal and verbal IQ, or the covariance between verbal IQ and more specific verbal tasks) is mainly due to genetic influences. Nonshared environmental influences primarily have a role in explaining test specific variance and have little effect on the overlap between abilities. These findings are in concordance with previous multivariate genetic studies into cognitive abilities (Petrill, 1997). The high genetic correlations across different cognitive domains suggest that there are "generalist genes": genes that exert general effects on various cognitive abilities (Kovas & Plomin, 2006; Plomin & Kovas, 2005). In both chapter 6 and 7, the genetic correlations between different cognitive abilities increased with age. This finding is in line with previous studies into specific cognitive abilities, that found low genetic correlations in infancy (Price et al., 2000), moderate correlations in childhood and adolescence (Alarcón et al., 1998; Alarcón et al., 1999) and high correlations in adulthood (Posthuma et al., 2001). These findings thus suggest progressive unidimensionality in cognitive abilities at the genetic level.

Various research groups have now started linkage and association studies and aim to find gene variants that influence cognitive abilities. Although some positive linkage and association results have been reported (see e.g. Dick et al., 2006; Dick et al., 2007; Luciano et al., 2006; Plomin, 2003; Posthuma et al., 2005; Posthuma & De Geus, 2006), most of the genetic variance to cognitive abilities still remains to be

elucidated. Plomin (2003) argues that the high heritability of intelligence is likely to underlie many DNA polymorphisms of small to vary small effect size. The application of recently developed genome wide association analyses (Craig & Plomin, 2006; Plomin et al., 2006) in combination with linkage studies may help clarify the precise role of genes in cognitive abilities. Several research groups have now also started to include specific measures of the environment in their data collection. These studies are needed to pinpoint down what aspects of the environment play a role in individual differences in cognition. Moreover, these studies may help elucidate the extent to which genetic and environmental effects correlate and how environmental factors can interact with genetic makeup (Posthuma & De Geus, 2006).

Testosterone levels and pubertal development

The last empirical chapter of this thesis described an examination of the sources of variation in testosterone levels in early adolescence and its covariation with pubertal development. Midday salivary testosterone levels were collected in 12-year-old twin pairs on two consecutive days. Furthermore, the twins were asked to fill out a questionnaire assessing their pubertal status. The analyses of pubertal development were restricted to those processes known to be under control of androgenic hormones: pubic hair development (boys and girls) and genital development (boys). The heritability of testosterone levels was 52% and was of equal magnitude in boys and girls. The remaining variance was explained by nonshared environmental effects. A relatively high correlation between testosterone levels in opposite sex twins was observed, suggesting an overlap in genetic expression in boys and girls. One previous study (Harris et al., 1998) in 14- to 21-year-old twins reported a near-zero correlation between plasma testosterone levels in opposite sex twins, indicating sex-specific genetic effects. Moreover, this study found no resemblance in testosterone concentrations between fathers and daughters and between mothers and sons. The participants in our study were younger, and most of the boys were still prepubescent. The results from our study suggests that the sex differences in gene expression found in later phases of development (Harris et al., 1998) have not yet (fully) developed in pre- and early puberty. Salivary testosterone levels correlated moderately with variation in androgen-dependent pubertal development (r=.31), this association was entirely explained by genetic factors.

Autistic traits and cognition

The main focus of this thesis was on the study of the aetiology of individuals differences in two domains: autistic traits and cognition. So far, the discussion of possible associations between autistic traits and cognition has been left untouched. In a review of epidemiological studies of pervasive developmental disorders (Fombonne,

2003), it was estimated that about 40% of the individuals with autism or another pervasive developmental disorder have severe to profound mental disabilities, 30% show mild to moderate impairments, while the remaining 30% shows normal or superior intelligence. Although autism spectrum condition (ASC) diagnoses are found in the entire range of intellectual abilities, various studies hint at a cognitive profile specific to ASCs.

Autism is often characterised by unevenly developed cognitive skills, which has most been studied using IQ profiles. Although an IQ profile with significantly lower verbal IQ than nonverbal IQ has been most strongly associated with autism (Lincoln et al., 1988), this profile is not universal in affected individuals. More recent studies suggest that the discrepancy between verbal and nonverbal IQ scores diminishes as intellectual ability approaches the normal range (Tager-Flusberg et al., 2001). Different cognitive theories have tried to describe the cognitive strengths and weaknesses of people with ASCs. The first theory posits that autism is mainly characterised by deficits in executive functioning (Hughes et al., 1994; Pennington & Ozonoff, 1996). Consistent with this theory is the finding that individuals with autism tend to perform poorly on tasks that tap executive functioning, such as the Wisconsin Card Sorting Test (Pennington & Ozonoff, 1996) and the Tower of Hanoi (Ozonoff et al., 1991), and on flexibility tasks such as the Verbal fluency test (Geurts et al., 2004). However, executive dysfunction is not specific to autism, but is also found in other domains of psychopathology, such as attention deficit hyperactivity disorder (Pennington & Ozonoff, 1996). The second theory trying to capture the cognitive profile of autism is the weak central coherence theory (Frith & Happe, 1994; Happe, 1999). This theory puts forward that autism is characterised by a cognitive style biased towards local, part-oriented processing, rather than global information processing. Consistent with this hypothesis is the relative peak performance on the Block design task (Happe, 1994; Siegel et al., 1996) and the Embedded figures task (Jolliffe & Baron-Cohen, 1997) found in individuals with autism. The latter findings are also in accordance with the third cognitive paradigm of autism, the extreme male brain theory (Baron-Cohen, 2002). This theory proposes that autism is characterised by impaired empathising (the drive to understand another's mental state and respond appropriately to it) and hyper-systemising skills (the drive to analyse a system in terms of rules to predict the behaviour of the system; Baron-Cohen et al., 2005; Baron-Cohen, 2006). Also consistent with this theory is the finding that people with Asperger syndrome perform worse on tests of emotion recognition (Baron-Cohen et al., 2001a) and social sensitivity (Baron-Cohen et al., 1999), but show intact or superior performance on tests of folk physics (Baron-Cohen et al., 2001c; Lawson et al., 2004).

Table 1. Partial correlations in both members of the twin between AQ scores and WAIS subtests and verbal fluency performance. All correlations are corrected for total IQ.

	AQ Oldest twin	AQ youngest twin
Vocabulary	-.05	-.08
Similarities	-.09	-.11
Information	.09	-.01
Picture completion	-.05	-.01
Block Design	**.19***	**.23****
Matrix reasoning	.08	.03
Arithmetic	.01	.00
Digit span	-.02	-.03
Letter-number sequencing	-.05	-.11
Digit - symbol coding	-.14	-.01
Symbol search	-.04	.02
Verbal fluency letters	**-.18***	**-.19***
Verbal fluency Categories	**-.16***	-.09

Note: * significant at p<.05 level; ** significant at p<.01 level

Studies in relatives of individuals diagnosed with an ASC can provide clues about the clustering of the cognitive profile of autism within families. Parents (Hughes et al., 1997; Piven & Palmer, 1997) and siblings (Ozonoff et al., 1993) of children with autism perform significantly worse on tests of executive functioning than controls. Siblings of children with autism show poor verbal fluency, but superior spatial and verbal span compared to siblings of children with developmental delay (Hughes et al., 1999). Parents of autistic probands also showed superior performance on the Embedded Figures Test and mildly impaired performance on an empathy test (Baron-Cohen & Hammer, 1997). No differences are found in working memory performance (Hughes et al., 1999), spatial-span memory (Hughes et al., 1997) or speed of information processing (Scheuffgen et al., 2000) in relatives of autistic probands.

These findings suggest that cognitive studies into autism may help us to better understand the autism phenotype, and that the cognitive profile clusters in families. As exemplified above, most research into the cognitive profile of ASCs has focused

on the cognitive style of autistic individuals themselves, or their relatives. Few studies have investigated the cognitive profile of autistic traits using a general population sample, and none have done so using a genetically informative design. I explored the association between scores on the AQ and the subtests of the WAIS in the 18-year-old twins who took part in my study. As previous studies suggested a negative association between autism and verbal fluency, tests of verbal fluency are also included in this analysis. The correlation between the AQ and total WAIS IQ is modest but significant (r=-.15). In order to examine the *relative* cognitive profile, instead of variance in general cognitive abilities per se, the correlations between AQ and WAIS subtests and verbal fluency are controlled for total IQ. These results are presented in Table 1.

Based on these phenotypic correlations, an interesting picture emerges. AQ scores are positively associated with performance on the block design task, and negatively associated with performance on verbal letter and category fluency. Even though the correlations are modest, they are in line with findings from studies in individuals with autism (Geurts et al., 2004; Happe, 1994; Siegel et al., 1996) and in relatives of autistic probands (Hughes et al., 1999). The positive association between AQ scores and performance on the block design task is in line with both the weak central coherence theory and the empathising systemising account of autism. The negative association with verbal fluency is in line with the executive dysfunction theory of autism, but could also be seen as congruent to the empathising systemising theory. Good empathising is likely to be related to frequent verbal interaction and may promote verbal fluency and vice versa. In the near future, further work on these data is planned, and I will explore whether the observed associations are due to common genetic effects. Ultimately, specifying the cognitive profile of the autism phenotype will advance our understanding of the heterogeneity of autism. Such analyses, together with molecular genetic, clinical, neurobiological, and imaging studies will hopefully lead to a better comprehension of the factors that make up the autism phenotype.

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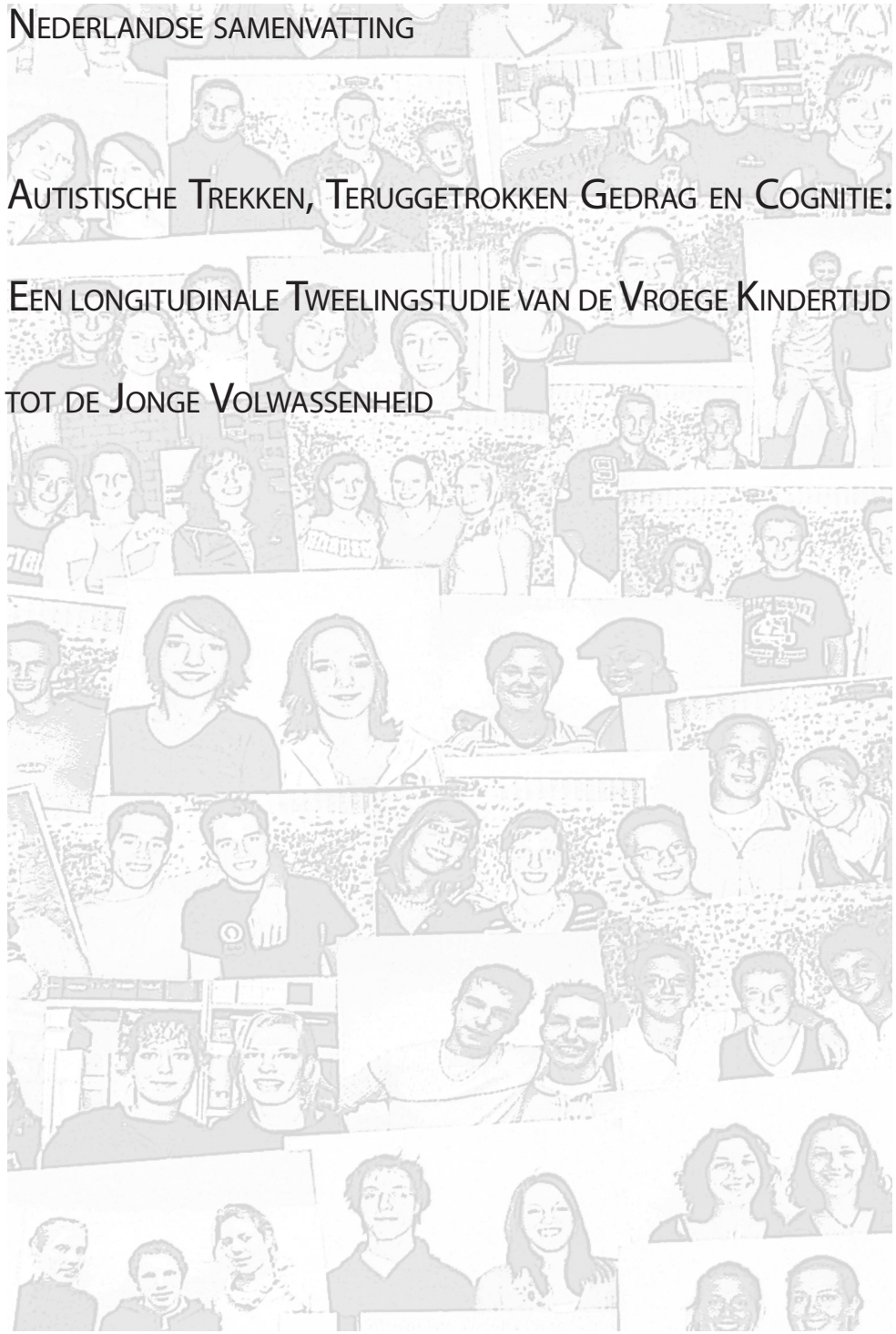
Nederlandse samenvatting



Autistische Trekken, Teruggetrokken Gedrag en Cognitie: Een longitudinale Tweelingstudie van de Vroege Kindertijd tot de Jonge Volwassenheid

Waarom hebben sommige jongeren veel moeite met sociale interactie, terwijl anderen juist het liefst de hele dag met vrienden optrekken? Welke factoren beïnvloeden individuele verschillen in teruggetrokken gedrag? In tweeling- en familieonderzoek kan op dit soort onderzoeksvragen worden ingegaan. Eeneiige tweelingen zijn genetisch identiek, terwijl twee-eiige tweelingen gemiddeld de helft van hun genetisch materiaal delen, net als gewone broers en zussen. Als eeneiige tweelingen van elkaar verschillen kan dit alleen worden veroorzaakt door omgevingsinvloeden. Verschillen tussen twee-eiige tweelingen kunnen zowel door omgevingsinvloeden als door verschillen in genetische aanleg worden veroorzaakt. Dit geldt ook voor gewone broers en zussen, maar zij zijn daarnaast ook nog op een ander moment geboren en opgegroeid. Door het vergelijken van de gelijkenis van eeneiige tweelingen en twee-eiige tweelingen en hun eenling broers en –zussen kan worden onderzocht in hoeverre genetische invloeden en omgevingsinvloeden van belang zijn bij het verklaren van verschillen tussen mensen in bijvoorbeeld gedrag of intelligentie (Boomsma et al., 2002). De omgevingsfactoren kunnen worden onderscheiden in twee soorten invloeden. De gedeelde, of gezinsinvloeden, zijn de invloeden die voor ieder in het gezin hetzelfde zijn en doen gezinsleden meer op elkaar lijken. Voorbeelden hiervan kunnen zijn de buurt waarin het gezin woont, de opvoeding, of bepaalde eetgewoonten. Daarnaast zijn er omgevingsinvloeden die voor ieder gezinslid uniek zijn, zoals hobby's, verschillende leraren op school, of verschillende vrienden. Deze invloeden zorgen ervoor dat gezinsleden van elkaar verschillen.

Dit proefschrift beschrijft de bevindingen van familieonderzoek en richt zich op twee domeinen. Ten eerste worden de oorzaken van individuele verschillen in autistische trekken en teruggetrokken gedrag onderzocht. Daarnaast worden de resultaten van een langlopende studie naar de ontwikkeling van cognitieve vaardigheden beschreven. Voor dit onderzoek zijn data verzameld in een groep van 209 tweelingparen die geregistreerd staan bij het Nederlands Tweelingen Register. Deze tweelingen worden al sinds 1992 gevolgd en hebben meegedaan aan uitgebreid intelligentieonderzoek op 5-, 7-, 10-, 12- en 18-jarige leeftijd. Toen de tweelingen 18 jaar oud waren werden ook hun broers en zussen uitgenodigd mee te doen aan het onderzoek. Tijdens de laatste meting hebben de tweelingen en hun broers en zussen ook aanvullende cognitieve tests gedaan en gedragsvragenlijsten ingevuld. Op zowel 12- als 18-jarige leeftijd hebben de jongeren daarnaast speeksel verzameld, waarin cortisol- en testosteronconcentraties konden worden gemeten en vulden zij een puberteitsvragenlijst in.

Individuele verschillen in autistische trekken

In hoofdstuk 2 tot en met 4 van dit proefschrift wordt ingegaan op de individuele verschillen in autistisch gedrag en op de vraag in hoeverre autistische trekken samenhangen met andere gedragsproblemen. In hoofdstuk 2 wordt een studie naar de betrouwbaarheid en validiteit van de Nederlandse vertaling van de Autisme-Spectrum Quotient (AQ; Baron-Cohen *et al.*, 2001) beschreven. Deze vragenlijst heeft tot doel het vóórkomen van autistische trekken te kwantificeren en is gebaseerd op zelfrapportage. De vragenlijst is afgenomen in een groep mensen uit de algemene populatie, een grote groep studenten en in 3 kleine patiëntgroepen, te weten bij personen met een diagnose voor een autisme spectrum stoornis, een sociale angststoornis, of een obsessief compulsieve stoornis. Scores op de AQ bleken normaal verdeeld in de studentenpopulatie en in de algemene populatie, met aan het linker uiteinde van de verdeling mensen met erg weinig autistische trekken en aan het rechter uiteinde mensen met extreem veel autistische gedragingen. De Nederlandse vertaling van de AQ heeft goede testeigenschappen, met een test-hertest betrouwbaarheid van .78 en een interne consistentie van α=.79. Mannen haalden gemiddeld een hogere AQ score dan vrouwen, dit komt overeen met resultaten uit eerder onderzoek waarin autistische trekken op een continue schaal zijn gemeten (Ronald et al., 2006; Wakabayashi et al., 2006) en is bovendien in lijn met de bevinding dat autisme vaker voorkomt bij mannen dan bij vrouwen (Fombonne, 2003). Studenten die een technische of natuurwetenschappelijke studie volgen scoorden significant hoger op de AQ dan studenten aan de letteren of sociale wetenschappen faculteit. Deze bevinding is in overeenstemming met eerder onderzoek waarin is aangetoond dat familieleden van autistische personen vaker een beroep in de technische of natuurwetenschappelijke sector uitoefenen (Baron-Cohen et al., 1997; Baron-Cohen et al., 2007) en dat studenten met ouders in een natuurwetenschappelijk of technisch beroep hoger scoren op de AQ dan hun studiegenoten met ouders in andere beroepssectoren (Austin, 2005). Patiënten met een autisme spectrum stoornis scoorden significant hoger op de AQ dan de andere twee patiëntgroepen. Dit is een bevredigend resultaat, omdat het laat zien dat een hoge AQ score specifiek is voor patiënten met een autisme spectrum diagnose en niet voor gedragsproblemen in het algemeen. Binnen de groep patiënten met een autisme spectrum stoornis behaalden mensen met de diagnose voor autisme of het syndroom van Asperger de hoogste scores. Patiënten met een pervasieve ontwikkelingsstoornis-niet anders omschreven (PDD-NOS, een brede diagnostische categorie, met minder stringente criteria dan de criteria voor een autisme stoornis diagnose (American Psychiatric Association, 2000)) behaalden een score die tussen de scores van de autisme en Asperger groep en de scores van de andere twee patiëntgroepen in lag. De andere twee patiëntgroepen scoorden weer hoger dan de algemene populatie. Deze resultaten wijzen erop dat autisme niet een duide-

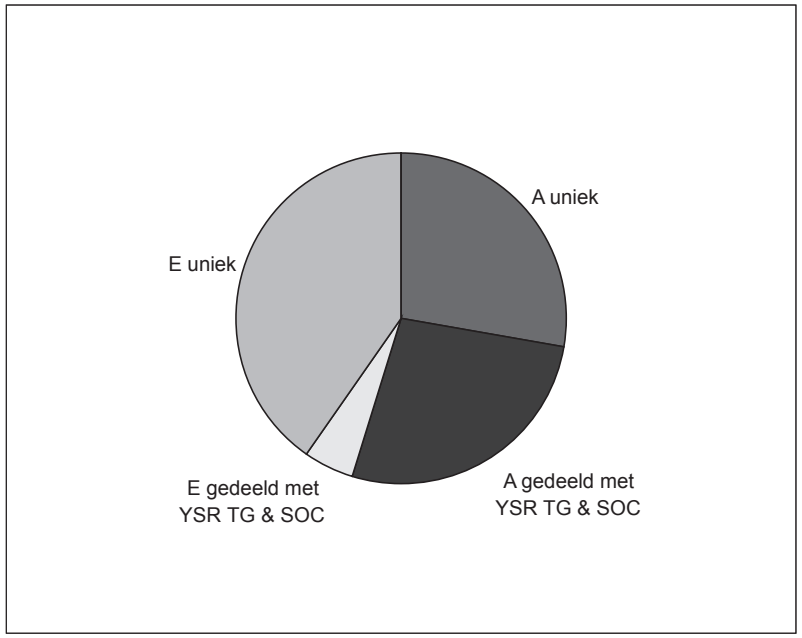
lijk te onderscheiden stoornis is die je wel of niet hebt, maar dat autistische trekken voorkomen in een spectrum en dat deze trekken een normaalverdeling volgen in de algemene populatie. De klinische diagnose autisme vormt in deze zienswijze het extreme uiteinde van de normaalverdeling. In hoofdstuk 2 is ook de factorstructuur van de AQ onderzocht. Met een factoranalyse kan worden bekeken of een vragenlijst één of juist meerdere dimensies (factoren) bestrijkt. In de AQ konden twee onderliggende factoren worden onderscheiden. De eerste factor bevat voornamelijk items die algemene problemen met sociale interactie weergeven. De tweede factor richt zich op een voorkeur en talent voor aandacht voor details. In het geheel laat hoofdstuk 2 zien dat de Nederlandse vertaling van de AQ een betrouwbaar instrument is om het vóórkomen van autistische trekken te meten.

Hoofdstuk 3 beschrijft een familiestudie naar de genetische en omgevingsinvloeden op individuele verschillen in autistische trekken. Voor dit onderzoek is de Nederlandse vertaling van de AQ afgenomen bij 18-jarige tweelingen en hun broers en zussen. Individuele verschillen in autistisch gedrag bleken in belangrijke mate door genetische factoren te worden beïnvloed: 57% van de variantie kon aan genetische effecten worden toegeschreven. De overige variantie werd verklaard door unieke omgevingsinvloeden. Jongen-meisje tweelingen leken even sterk op elkaar als twee-eiige tweelingen van hetzelfde geslacht. Dit patroon wijst erop dat er geen sekse-specifieke genen zijn die de variantie in autistische trekken beïnvloeden. Tweelingen hebben niet meer of minder autistische trekken dan hun niet-tweeling broers en zussen, hun gemiddelde AQ scores waren gelijk. Eerder tweelingonderzoek heeft uitgewezen dat variatie in autistische trekken redelijk tot sterk erfelijk bepaald is in de kindertijd en vroege adolescentie (Constantino & Todd, 2000; Constantino & Todd, 2003; Ronald et al., 2005; Ronald et al., 2006). Het onderzoek in hoofdstuk 3 biedt een uitbreiding op dit onderzoek en laat zien dat deze eigenschappen ook onder genetische controle staan in de late adolescentie. Ten slotte is in hoofdstuk 3 onderzocht of mensen bij het kiezen van hun partner rekening houden met de autistische trekken van hun partner. Als mensen actief danwel passief op zoek gaan naar een partner met vergelijkbaar autistisch gedrag ("soort zoekt soort") of juist naar een partner met tegenovergesteld gedrag ("tegenpolen trekken elkaar aan"), zou dit de resultaten uit ons tweelingonderzoek kunnen vertekenen. De AQ scores van 128 partners uit de algemene bevolking (allen getrouwd of samenwonend) werden met elkaar vergeleken. De correlatie tussen de partners (een maat voor de samenhang, een correlatie (r) van 0 wijst op de afwezigheid van samenhang, r=1 weerspiegelt een perfecte overeenkomst) was erg laag in niet significant (r=.05, p=.59) en indiceert dat er geen actieve danwel passieve partnerselectie is voor autistische trekken.

Hoofdstuk 4 beschrijft een onderzoek naar de covariantie tussen autistische trekken en andere gedrags- en emotionele problemen. Hiervoor werden, behalve AQ

data, ook gegevens over gedragsproblemen verzameld, door een grote groep 18-jarige tweelingen en hun broers en zussen te vragen de Youth Self Report vragenlijst (YSR; Achenbach & Rescorla, 2001; Verhulst et al., 1997) in te vullen. De YSR is een zelfrapportage vragenlijst en bestaat uit 8 syndroomschalen. Met stepwise backward regressie analyse is onderzocht welke YSR schalen een significante samenhang vertoonden met AQ scores. De schalen teruggetrokken gedrag (TG) en sociale problemen (SOC) bleken significante voorspellers en verklaarden, met sekse als derde predictor, 23% van de variantie in autistische trekken. Multivariaat genetische modellen wezen uit dat de overlap tussen de YSR schalen en de AQ voornamelijk toe te schrijven is aan genetische effecten. Ongeveer de helft van de genetische variantie in AQ scores was gedeeld met variantie in YSR TG en SOC scores. Dit wijst erop dat er overlappende genen zijn die beide gedragingen beïnvloeden. De overige genetische variantie was ongedeeld met de YSR schalen. Dit duidt er op dat er ook een set genen is die specifiek het voorkomen van autistische trekken beïnvloedt en geen effect heeft op ander probleemgedrag. De unieke omgevingsinvloeden op de AQ waren grotendeels specifiek, slechts 11% van de unieke omgevingsvariantie liet een overlap zien met de YSR scores. Dit hoofdstuk laat zien dat individuele verschillen in autistische trekken, als gemeten in een algemene populatiegroep, samenhangen met sociaal en teruggetrokken gedragsproblemen en dat deze problemen deels door eenzelfde set genen worden beïnvloed.

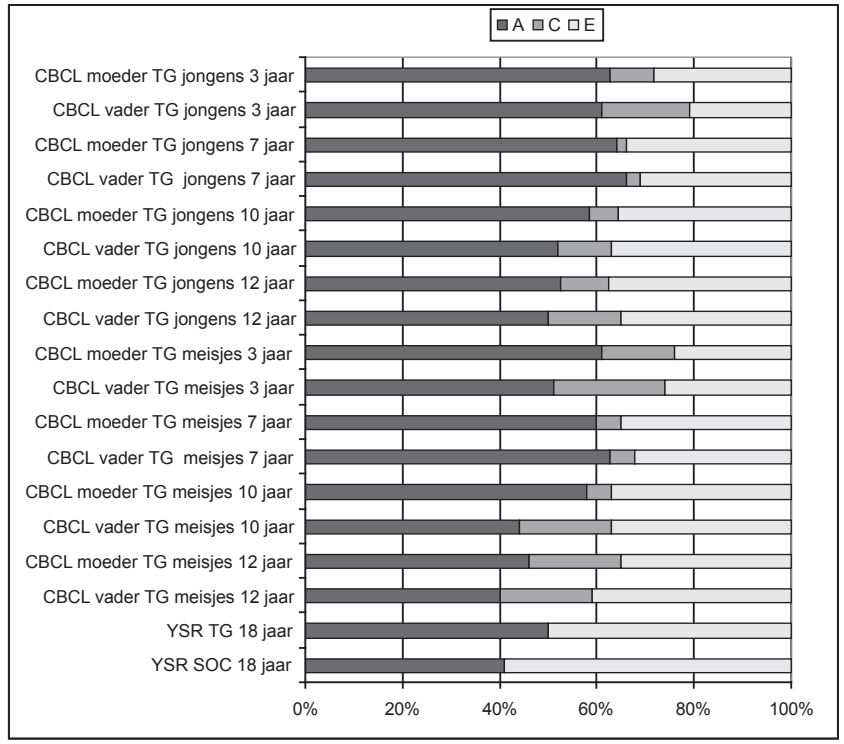
In figuur 1 worden de verschillende bronnen van variantie in autistische trekken samengevat. Additief genetische invloeden (A) verklaren 57% van de variantie in autistische trekken in de late adolescentie. De helft van deze genetische variantie wordt gedeeld met genetische variantie in teruggetrokken gedrag en sociale gedragsproblemen. De overige variantie wordt verklaard door unieke omgevingsinvloeden (E), waarvan een klein deel ook een invloed heeft op teruggetrokken en sociale gedragsproblemen. De test-hertest betrouwbaarheid van de AQ was .78. Dit duidt erop dat 39% ($(1 - .78^2)*100\% = 39\%$) van de variantie in AQ scores niet stabiel is en mogelijk toegeschreven kan worden aan meetfouten. De invloed van meetfouten op de variantie wordt weergegeven in de unieke omgevingsfactoren. Een mogelijke meetfout van 39% laat dan nog maar weinig extra ruimte over voor "echte" unieke omgevingsinvloeden. Deze resultaten wijzen uit dat niet alleen de klinische diagnose sterk erfelijk is, maar dat genetische factoren ook een belangrijke rol spelen bij het verklaren van individuele verschillen in autistisch gedrag in de algemene populatie. Deze bevindingen zijn ook belangrijk voor linkage en associatie studies (onderzoek naar de specifieke genen betrokken bij autistisch gedrag). In plaats van een stringente scheiding van aangedane en niet-aangedane personen is het misschien beter om autistische trekken op een kwantitatieve schaal te meten en zo rekening te houden met de variatie in autistische trekken.



Figuur 1. Overzicht van de verschillende bronnen van variantie in autistische trekken.
Noot: A = additieve genetische effecten; E = unieke omgevingsinvloeden; SOC = sociale gedragsproblemen; TG = teruggetrokken gedrag; YSR = Youth Self Report.

Teruggetrokken gedrag

Hoofdstuk 5 gaat over een langlopende studie naar teruggetrokken gedrag in de kindertijd. Voor dit onderzoek zijn zowel vaders als moeders gevraagd het teruggetrokken gedrag van hun tweeling te beoordelen toen de tweelingen 3, 7, 10 en 12 jaar oud waren. De mate van teruggetrokken gedrag werd gemeten met de schaal "teruggetrokken gedrag" uit de Child Behavior Checklist vragenlijst (CBCL; Achenbach & Rescorla, 2001; Verhulst et al., 1996). Omdat ouders kunnen verschillen in hun beoordeling van het gedrag van hun kinderen, zijn zowel de vader- als moederrapportages geanalyseerd. Zo kon een onderscheid worden gemaakt tussen beoordelaarspecifieke effecten en gedrag dat door beide beoordelaars werden opgemerkt. Individuele verschillen in teruggetrokken gedrag in de kindertijd bleken in belangrijke mate te kunnen worden toegeschreven aan genetische factoren (zie figuur 2). Genetische effecten verklaarden 50 tot 66% van de variantie in teruggetrokken gedrag tussen leeftijd 3 en 12 jaar in jongens en 40 tot 61% van de variantie in meisjes. Gedeelde omgevingsinvloeden (C) verklaarden een klein maar wel significant deel van de variantie (2-23%) en waren iets belangrijker in meisjes dan in jongens. Unieke omgevingsinvloeden verklaarden 21 tot 41% van de variantie in beide seksen en op alle leeftijden.



Figuur 2. Percentage van de totale variantie dat wordt verklaard door additief genetische invloeden (A), gedeelde omgevingsinvloeden (C) en unieke omgevingsinvloeden (E) in teruggetrokken gedrag (TG) en sociale problemen (SOC) op verschillende momenten in de ontwikkeling.
Noot: CBCL = Child Behavior Checklist; YSR = Youth Self Report.

Met langlopend tweelingonderzoek kan ook worden onderzocht in hoeverre genetische of omgevingsinvloeden van belang zijn voor de continuïteit van gedrag. Teruggetrokken gedrag is een vrij stabiele eigenschap, de samenhang tussen de teruggetrokken gedragscores op verschillende leeftijden varieerde van .23 tot .29 voor de stabiliteit tussen leeftijd 3 en 12 jaar, tot een correlatie van .65 voor de stabiliteit tussen leeftijd 10 en 12 jaar. Genetische factoren waren erg belangrijk voor het verklaren van stabiliteit van teruggetrokken gedrag en verklaarden 74% van de stabiliteit in jongens en 65% van de stabiliteit in meisjes. Gedeelde omgevingsinvloeden waren ook van significant belang en verklaarden 8% van de stabiliteit in jongens en 18% van de stabiliteit in meisjes. De ouders waren het over het algemeen eens over het vóórkomen van het gedrag bij hun kind, wat erop wijst dat dit "echte" gedeelde omgevingsinvloeden zijn, en geen vertekening door het perspectief van de beoordelaar. Unieke omgevingsinvloeden verklaarden de rest van de continuïteit in teruggetrokken gedrag. Ook hier waren beide beoordelaars het meestal met elkaar eens, wat

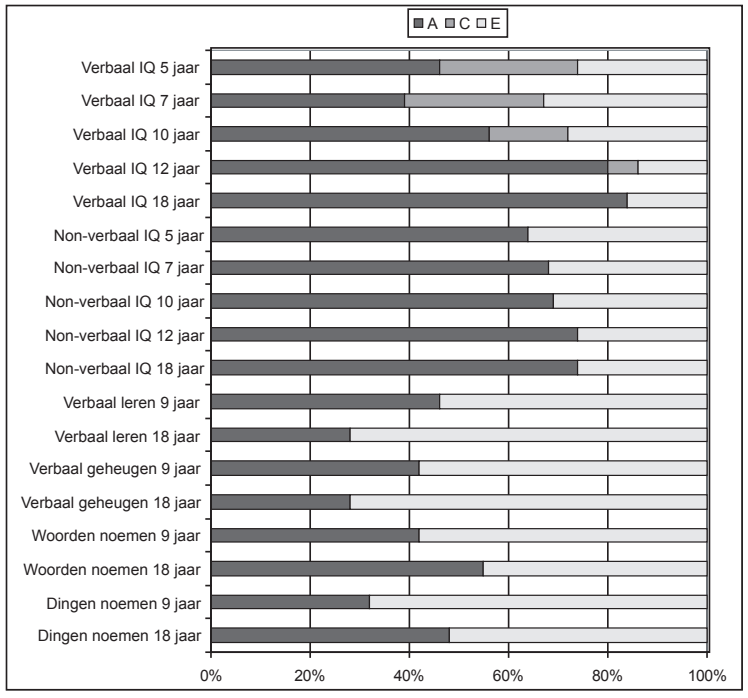
aangeeft dat dit ware effecten zijn en niet zijn toe te schrijven aan onbetrouwbaarheid van de meetschaal. Uit enkele eerdere onderzoeken blijkt dat de stijl van opvoeden (bijvoorbeeld te sterke controle door de ouder) de stabiliteit van teruggetrokken gedrag kan beïnvloeden in jonge kinderen (Park et al., 1997; Rubin et al., 2002). Als de ouder deze opvoedingsstijl bij beide kinderen hanteert, is dit een gedeelde omgevingsinvloed. Een kindspecifieke opvoedingsstijl zou worden weergegeven in de unieke omgevingsinvloeden. De correlaties tussen genetische en gedeelde omgevingsinvloeden over de jaren heen waren erg hoog. Dit wijst erop dat de set genen en de gedeelde omgevingsinvloeden die teruggetrokken gedrag door de kinderjaren heen beïnvloeden vrijwel dezelfde blijven.

Het onderzoek naar de YSR zelfrapportage van teruggetrokken gedrag in 18-jarige tweelingen en hun broers en zussen (behandeld in hoofdstuk 4) geeft een beeld van het belang van genetische en omgevingsfactoren op teruggetrokken gedrag in de late adolescentie. De erfelijkheid van teruggetrokken gedrag werd geschat op 50%. Dit laat zien dat genen een belangrijke factor blijven voor het verklaren van individuele verschillen in teruggetrokken gedrag in een latere fase van de ontwikkeling. Gedeelde omgevingsinvloeden bleken niet van belang op deze leeftijd. Het aantal families betrokken in het onderzoek in de late adolescentie (424 families) is aanzienlijk lager dan het aantal families dat deelnam aan het onderzoek in de kindertijd (14.889). Het zou dan ook kunnen dat het onderzoek in de 18-jarigen te weinig statistische power had om gedeelde omgevingsinvloeden te kunnen detecteren. Echter, de tweelingcorrelaties voor teruggetrokken gedrag op 18-jarige leeftijd (r=.55 in eeneiige tweelingen; r=.28 in twee-eiige tweelingen) geven geen indicatie voor gedeelde omgevingsinvloeden op deze leeftijd. Hoewel de meeste tweelingen en hun broers en zussen nog bij hun ouders woonden ten tijde van het onderzoek (92% volgens de data uit ons vragenlijstonderzoek), lijken de omgevingsinvloeden op teruggetrokken gedrag dus vooral ongedeeld met de andere familieleden. Gedurende de adolescentie brengen westerse jongeren meer en meer tijd door op school en met vrienden en minder tijd binnen het gezin (Larson & Verma, 1999). Dit zou kunnen verklaren waarom de gedeelde omgevingsinvloeden op teruggetrokken gedrag verwaarloosbaar zijn in de late adolescentie.

In hoofdstuk 4 is ook gekeken naar de genetische en omgevingsinvloeden op zelfgerapporteerde sociale problemen. Genetische invloeden verklaarden 41% van de variantie, het grootste deel van de variantie (59%) werd verklaard door unieke omgevingsinvloeden. Het Nederlands Tweelingen Register verzameld sinds enkele jaren ook gegevens over gedragsproblemen wanneer de tweelingen 14 en 16 jaar oud zijn. Hiermee komen in de toekomst data beschikbaar van kinderen die van 3 tot 18-jarige leeftijd op meerdere meetmomenten in de kindertijd en de adolescentie zijn gevolgd en wordt het mogelijk de stabiliteit van teruggetrokken gedrag en sociale gedragsproblemen in de adolescentie te onderzoeken.

Cognitieve vaardigheden

Binnen het onderzoek in de gedragsgenetica is relatief veel aandacht voor cognitieve vaardigheden. Er zijn in het verleden al veel onderzoeken gedaan naar de erfelijkheid van verschillende cognitieve vaardigheden en naar de ontwikkeling van cognitieve vaardigheden in de kindertijd. Tot nu toe heeft echter geen enkel onderzoek de ontwikkeling van cognitieve vaardigheden gevolgd van de kindertijd tot in de volwassenheid. In dit proefschrift wordt ingegaan op de ontwikkeling van verbale en non-verbale intelligentie van de vroege kindertijd tot de jonge volwassenheid en worden de genetische en omgevingsinvloeden op de overlap tussen verschillende verbale vaardigheden onderzocht op twee verschillende momenten in de ontwikkeling. In hoofdstuk 6 wordt een longitudinale studie beschreven waarin IQ data zijn verzameld in tweelingen toen zij 5, 7, 10, 12 en 18 jaar oud waren. Zowel verbaal als non-verbaal IQ was erg stabiel over de tijd, met correlatie coëfficiënten variërend van .47 tussen leeftijd 5 en 18 jaar, tot .80 voor kortere tijdsintervallen. Net als in eerdere studies (Bartels et al., 2002; Bishop et al., 2003; Petrill et al., 2004; Posthuma et al., 2002) bleek uit de multivariate longitudinale genetische analyses dat genetische invloeden op intelligentie toenemen met de leeftijd. Genetische invloeden verklaarden 48% (verbaal IQ) en 64% (non-verbaal IQ) van de variantie op 5-jarige leeftijd en 84% (verbaal IQ) en 74% (non-verbaal IQ) op 18-jarige leeftijd (zie figuur 3). Gedeelde omgevingsinvloeden waren alleen van belang voor het verklaren van individuele verschillen in verbaal IQ. In overeenstemming met eerder onderzoek namen deze invloeden af in de loop van de ontwikkeling en waren ze niet langer significant in de jonge volwassenheid. Genetische effecten waren het belangrijkst voor het verklaren van de stabiliteit van intelligentie. De continuïteit van non-verbaal IQ werd geheel verklaard door genetische factoren. Stabiliteit in verbale intelligentie werd verklaard door sterke genetische effecten en matige gedeelde omgevingsinvloeden. De gedeelde omgevingsinvloeden waren alleen van belang voor de stabiliteit in de vroege kindertijd, na 10-jarige leeftijd waren deze effecten niet meer van belang. Unieke omgevingsinvloeden hadden alleen tijdelijke effecten en droegen niet bij aan de stabiliteit van verbale en non-verbale intelligentie. De positieve samenhang tussen verbaal en non-verbaal IQ werd geheel verklaard door genetische effecten. De genetische correlatie tussen deze twee maten nam toe met de jaren, van .62 op 5-jarige leeftijd tot .73 op 18-jarige leeftijd. Dit correlatiepatroon suggereert dat de genetische overlap tussen verschillende cognitieve vaardigheden toeneemt in een latere fase van ontwikkeling.



Figuur 3. Percentage van de totale variantie dat wordt verklaard door additief genetische invloeden (A), gedeelde omgevingsinvloeden (C) en unieke omgevingsinvloeden (E) in verbaal en non-verbaal IQ en verschillende verbale vaardigheden op verschillende momenten in de ontwikkeling.

In hoofdstuk 7 wordt ingegaan op de erfelijkheid van verschillende verbale vaardigheden in de kindertijd en late adolescentie. In twee groepen tweelingfamilies, bestaande uit 9-jarige of 18-jarige tweelingen en hun broers en zussen, werden verschillende verbale tests afgenomen. Verbaal IQ werd bepaald met een uitgebreide intelligentietest, daarnaast werden tests afgenomen die verbaal leren, verbaal geheugen en spontane woordproductie meten. Verbaal leren en geheugen werd gemeten door de proefpersoon te vragen zoveel mogelijk woorden van een lijst te onthouden en deze meerdere keren te reproduceren. Om de spontane woordproductie te meten moesten de proefpersonen zoveel mogelijk woorden noemen beginnend met een bepaalde letter ("woorden noemen") of behorend tot een bepaalde categorie ("dingen noemen"). In dit onderzoek kon de erfelijkheid van verschillende verbale vaardigheden worden onderzocht. Ook is bekeken in hoeverre de verschillende verbale vaardigheden met elkaar samenhangen en of deze overlap door genetische of door omgevingsinvloeden wordt veroorzaakt. Omdat het onderzoek is uitgevoerd op twee verschillende leeftijden, kon ook worden bekeken of de genetische en omgevingsinvloeden anders zijn in deze verschillende fasen van ontwikkeling. In beide onderzoeksgroepen bleken

twee-eiige tweelingen evenveel op elkaar te lijken als tweelingen op hun niet-tweeling broers en zussen. Dit wijst erop dat er geen tweelingspecifieke factoren een rol spelen bij individuele verschillen in verbale vaardigheden. De erfelijkheid van verbaal IQ was hoog in beide cohorten (82 en 84%), de erfelijkheid van de andere verbale maten was lager en varieerde van 28 tot 55% (zie figuur 3). Gedeelde omgevingsinvloeden waren niet van belang, noch in de kindertijd noch in de late adolescentie. Tegen de verwachting in was de erfelijkheid van verbaal leren en geheugen niet hoger in het oudere cohort. Waarschijnlijk komt dit doordat de taak waarmee verbaal leren en geheugen werd gemeten iets verschilde tussen de verschillende cohorten. De jonge kinderen moesten ongerelateerde woorden onthouden, terwijl de oudere groep gevraagd werd woorden uit 4 categorieën te onthouden. Eerder onderzoek heeft laten zien dat het leervermogen voor ongerelateerde woorden sterker erfelijk is dan het leren van gecategoriseerde woorden (Volk et al., 2006). Dit zou de relatief lage erfelijkheid van verbaal leren in het oudere cohort kunnen verklaren. De erfelijkheid van woordproductie was wel hoger in de adolescentie, dit gold voor zowel woorden noemen als dingen noemen. In beide cohorten werd de samenhang tussen de verschillende tests vooral verklaard door genetische factoren (70 en 74%), de overige covariantie werd verklaard door unieke omgevingsfactoren. Het voornaamste verschil tussen beide cohorten betrof de mate van samenhang tussen de verschillende verbale tests. De overlap was groter in het adolescenten cohort, zowel de samenhang tussen de tests zelf (de fenotypische correlatie) als de samenhang tussen de genetische factoren die de tests beïnvloeden (de genetische correlatie). De samenhang tussen verbale vaardigheden lijkt dus toe te nemen in een latere fase van ontwikkeling.

Het algemene beeld dat uit het onderzoek besproken in hoofdstuk 6 en 7 naar voren komt is dat de covariantie tussen verschillende cognitieve vaardigheden (zowel de samenhang tussen verbaal en non-verbaal IQ als de samenhang tussen verbaal IQ en meer specifieke verbale vaardigheden) vooral wordt veroorzaakt door genetische factoren. Unieke omgevingsinvloeden daarentegen zijn vooral van belang in het verklaren van testspecifieke variantie en hebben weinig effect op de samenhang tussen verschillende vaardigheden. De hoge genetische correlaties tussen verschillende cognitieve vaardigheden duiden op het bestaan van "algemene cognitieve genen": genen die een brede invloed uitoefenen op verschillende cognitieve domeinen (Kovas & Plomin, 2006; Plomin & Kovas, 2005). De genetische correlaties tussen de verschillende tests namen toe met de leeftijd, zowel in het onderzoek in hoofdstuk 6 als in de studie beschreven in hoofdstuk 7. Enkele eerdere onderzoeken vonden ook lage genetische correlaties in heel jonge kinderen (Price et al., 2000), matige correlaties in de kindertijd en adolescentie (Alarcón et al., 1998; Alarcón et al., 1999) en hoge correlaties in de volwassenheid (Posthuma et al., 2001). Gecombineerd duiden deze resultaten op een toenemende samenhang tussen cognitieve vaardigheden op

genetisch niveau: terwijl in de jonge kindertijd de genen voor verschillende vaardigheden nog erg verschillen, lijkt in de jonge volwassenheid een meer eenduidige set genen verschillende vaardigheden te beïnvloeden.

Testosteron en puberteitsontwikkeling

In het laatste empirische hoofdstuk van dit proefschrift wordt ingegaan op de genetische en omgevingsinvloeden op testosteronniveaus in de vroege adolescentie en de samenhang tussen testosteronniveaus en puberteitsontwikkeling. Hiervoor is bij 12-jarige tweelingen het testosteronniveau gemeten in speekselmonsters en is hen gevraagd een puberteitsvragenlijst in te vullen om te meten hoe ver zij in de puberteit zijn. We richtten ons op de testosteronafhankelijke puberteitsontwikkeling: de ontwikkeling van schaamhaargroei (zowel jongens als meisjes) en de ontwikkeling van de genitaliën (alleen jongens). De genetische invloeden op individuele verschillen in testosteronniveaus waren even groot in meisjes als in jongens (52%). De rest van de variantie werd verklaard door unieke omgevingsinvloeden. De correlatie tussen testosteronniveaus in jongen-meisje tweelingen was relatief hoog en duidt erop dat (een deel van) de genen die testosteronniveaus beïnvloeden hetzelfde is in jongens en meisjes op deze leeftijd. In een eerdere studie in een oudere onderzoeksgroep (14 tot 21-jarigen) werd vrijwel geen samenhang gevonden tussen de testosteronniveaus van man-vrouw tweelingen en tussen de testosteronconcentraties van vaders en hun dochters en moeders en hun zonen (Harris et al., 1998). De resultaten uit ons onderzoek wijzen erop dat de sekseverschillen in genexpressie nog niet vol van kracht zijn in de vroege en prepuberteit. De testosteronniveaus als gemeten in het speeksel hingen positief samen met de puberteitsontwikkeling (r=.31), deze samenhang werd geheel door genetische invloeden verklaard.

Ten slotte

Gebaseerd op de resultaten van de onderzoeken die in dit proefschrift zijn beschreven, kan worden vastgesteld dat genetische invloeden een belangrijke rol spelen tijdens de ontwikkeling van de vroege kindertijd tot de jonge volwassenheid. Genen beïnvloeden individuele verschillen in autistische trekken, teruggetrokken gedrag en cognitieve vaardigheden in verschillende fasen van ontwikkeling. Bovendien zijn genen van groot belang voor de stabiliteit van gedrag en cognitie. Gedeelde omgevingsinvloeden zijn vooral van belang in de kindertijd, terwijl unieke omgevingsinvloeden op alle leeftijden vooral een tijdelijke invloed hebben en niet of weinig bijdragen aan de stabiliteit van gedrag en cognitie over tijd. Dit onderzoek laat tevens zien dat het de moeite waard is gedragsproblemen in een niet-klinische groep te onderzoeken. Patiënten met een diagnose voor een autisme spectrum stoornis vormen een erg heterogene groep, met zeer verschillend functioneringsniveau en ernst van de stoornis. Inzicht in de variatie van autistische trekken in de algemene populatie en in de genetische en omgevingsinvloeden op deze variatie kan ons mogelijk veel leren over de klinische stoornis zelf.

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APPENDICES

APPENDIX I:

Sample Characteristics and Data collection

Participants and procedures – longitudinal data collection

This thesis reports on the results from a longitudinal study into the development of intelligence and problem behaviour. The study was initiated in 1992 with the recruitment of 209 5-year-old twin pairs from the Netherlands Twin register (NTR), kept by the Department of Biological Psychology at the VU University in Amsterdam (Bartels et al., 2007; Boomsma et al., 2006). An overview of the longitudinal data collection is presented in Table 1. The twin families were selected on the basis of age, zygosity of the twins, and their place of residence. At age 5 years (mean age 5.3 years, sd=0.2), the twins completed the short version of the Revised Amsterdamse Kinder Intelligentie Test (RAKIT; Bleichrodt et al., 1984) and participated in an electrophysiological experiment (Boomsma & Van Baal, 1998; Van Baal, 1997; Van Baal et al., 2001). At the second measurement occasion (mean age 6.8 years, sd=0.2) 192 pairs of the initial sample completed the test protocol, which again comprised the RAKIT and an electrophysiological experiment. The first two measurement occasions both took place at the VU University laboratory. At the third data collection (mean age 10.0 years, sd=0.1) 197 twin pairs of the original sample participated, and all completed the RAKIT (Rietveld, 2003). The fourth assessment was conducted when the twins were 12 years old (mean age 12.0 years, sd=0.1) and was completed by 192 twin pairs. This time, the twins were asked to complete the Wechsler Intelligence Scale for Children-Revised (WISC-R; Van Haassen et al., 1986) and to collect saliva samples for hormone assessment (Bartels, 2003). For the third and forth data collection, the tests took place either at the twins' home or at the VU University, depending on the preference of the twin family. The majority of the families preferred testing at home (around 70% of the families at both time points). Six years after the fourth measurement occasion, 122 twin pairs of the initial sample participated in the fifth assessment (mean age 18.1 years, sd=0.2). This assessment comprised an extensive test protocol including both physical measurements (carried out by a paediatrician) and a psychological test protocol. The findings of the psychological test protocol are reported on in this thesis. To increase the sample size on the fifth assessment, 64 additional twin (mean age 18.3 years, sd=0.1) families were recruited via the NTR. At the fifth measurement occasion, the siblings of the twins were also asked to take part. In total, 101 siblings participated (48 brothers and 53 sisters, mean age 18.6 years, sd=4.8), coming from 93 families (in 8 cases 2 siblings from the same family took part). Complete data on all 5 measurement occasions were available for 115 twin pairs.

TABLE 1. Overview of the longitudinal data collection at age 5, 7, 10, 12 and 18 years.

	N twin pairs	Brain activity	Other	Cognition	Questionnaires	Hormones (saliva)
Age 5	209	EEG ERP	Heart rate	RAKIT	*Behavioural:* Parents: Devereux	
Age 7	192	EEG ERP	Heart rate	RAKIT	*Behavioural:* Parents: CBCL	
Age 10	197			RAKIT	*Behavioural:* Parents: CBCL	
Age 12	192			WISC-R	*Behavioural:* Parents: CBCL, educational achievement Teachers: TRF, educational achievement Children: YSR *Pubertal development:* Children: Tanner	Cortisol Testosterone
Age 18	122+64*		Heart rate	WAIS-III	*Behavioural:* Children: YSR *Pubertal development:* Children: Tanner	Cortisol Testosterone

* 122 families from the longitudinal sample and 64 newly recruited families

Of all twin pairs from the longitudinal sample, 42 were monozygotic males (MZM), 44 were dizygotic males (DZM), 47 monozygotic females (MZF), 37 dizygotic females (DZF), and 39 dizygotic twin pairs of opposite sex (DOS). For the same sex twin pairs, zygosity was based on blood group polymorphisms (52 pairs) or DNA analyses (111 pairs). For the remaining twins, zygosity was determined by physical resemblance assessed by an experienced test administrator (4 pairs) or by discriminant analyses of longitudinally collected questionnaire items (3 pairs; Rietveld et al., 2000). Of all newly recruited families that only participated at age 18, there were 13 MZM twin pairs, 12 DZM pairs, 16 MZF pairs, 9 DZF pairs and 14 DOS twin pairs. Zygosity determination in the same sex twins of this group was based on DNA analysis (47 pairs), blood group polymorphisms (2 pairs) or questionnaire items (1 pair).

Non-responders

Up to the fourth measurement occasion, the drop-out in the study was low, as 92% of the original families still participated. At the fifth time point, the choice to participate was no longer made by the parents, but by the twins and siblings themselves and participation rate decreased to 58%. For the majority of families, lack of time

TABLE 2. Test protocol and approximate starting time of the different tests.

Programme testing day	Starting time	Remarks
Start medical test protocol at VU medical centre	10.00 AM	All together
Attach electrodes + VU-AMS device	10.05	All together
Blood pressure	10.30	All together
Blood sample	10.30	All together
Food + drinks	10.40	All together
Physical examination (simultaneously the other participants wait in the corridor and fill out the Autism-Spectrum Quotient)	10.45	Individually
Wait in the corridor, fill out the Autism-Spectrum Quotient (while the other participants are being examined)	11.10	Individually
Blood pressure	11.45	Individually
Lunch break	12.00	All together
Start psychological test protocol at VU psychology test laboratory		
Collection questionnaires, saliva measures and DNA samples	12.30	All together
Blood pressure (simultaneously one of the other participants completes finger print scan)	12.40	All together
Finger print scan (simultaneously the other participants have their blood pressure taken)	12.40	All together
Wechsler IQ	13.00	Individually
Tea break	14.30	All together
Stroop	14.45	Individually
Verbal Fluency	14.50	Individually
Box marking test	14.55	Individually
CVLT part 1	15.58	Individually
Reading the mind in the eyes	15.15	Individually
Blood pressure	15.25	Individually
Π inspection time task	15.28	Individually
CVLT part 2	15.35	Individually
n-back task	15.40	Individually
Corsi block tapping task	16.00	Individually
End test protocol	16.10	
Participants leave VU University	±16.20	

or difficulties to take leave from work or school were the prime reason to no longer take part. No significant differences in verbal and nonverbal IQ at age 5 were found for subjects who did not wish to participate in one of the assessments at age 7, 10 or 12 years ($F(3, 203)=.663$, $p=.576$ for verbal IQ; $F(3, 205)=1.660$, $p=.177$ for nonverbal IQ). However, subjects who continued to participate at age 18 had higher mean verbal ($F(1, 205)=7.834$, $p=.006$, $d=.40$) and nonverbal ($F(1, 207)=4.471$, $p=.036$, $d=.30$) IQ scores at age 5 as compared to subjects who did no longer take part when they were 18 years old. The parental education was slightly higher in the families who participated in the fifth measurement occasion, compared to the nonparticipating families (maternal education, $U=9538.00$, $p=.05$, effect size $r=.12$; paternal education, $U=7773.00$, $p=.01$, $r=.16$).

Data collection at the fifth measurement occasion

The fifth data collection, when the twins were 18 years of age, took place between 24 November 2004 and 19 July 2006. The testing day consisted of two parts: In the morning, the twins and their siblings completed the medical test protocol carried out by a paediatrician at the VU medical centre. After lunch, the participants completed the psychological test protocol. The complete programme of the testing day and the approximate starting times of the different tests are provided in Table 2.

Medical test protocol

After the twin families arrived at the VU medical centre, the testing day began by attaching the Vrije Universiteit Ambulatory Monitoring System (VU-AMS) to the body of each participating family member. The VU-AMS is a device to ambulatory record electrocardiogram and impedance cardiogram (De Geus et al., 1995; Willemsen et al., 1996). This way, cardiovascular functioning could be measured throughout the testing day. Next, blood pressure was assessed and a series of blood samples was taken. After the blood samples were collected, the participants received some food and drinks. Subsequently, each participant underwent a physical examination. The paediatrician first asked questions about their general condition, smoking and drinking behaviour, and their pubertal development. The girls were asked about their menarche and about the frequency and regularity of their period. Next, the paediatrician conducted a series of measures of the physical development of the participant, such as height, weight, head circumference, and skin folds. To ensure privacy during the physical examination, the other family members were asked to leave the testing room and to fill out the Autism-Spectrum Quotient in the meantime. After each participant had completed the physical test protocol, the twin families had lunch at the university restaurant.

TABLE 3. Overview of all measures assessed as part of the psychological test protocol in 18-year-old twins and their siblings.

Phenotype	Task/measure	N total	N twins*	N siblings	N VU	N home
Cognition						
Intelligence	Wechsler IQ	467	366 (241/125)	101	467	-
Inhibition	Stroop	465	364 (239/125)	101	465	-
Verbal fluency	Letter & category fluency	464	363 (238/125)	101	464	-
Verbal learning and memory	CVLT	463	364 (239/125)	99	463	-
Theory of mind	Reading the mind in the eyes	461	365 (240/125)	96	461	-
Perceptual speed	Π inspection time task	456	357 (232/125)	99	456	-
Working memory	n-back task	451	353 (230/123)	98	451	-
Spatial memory	Corsi block tapping task	463	362 (238/124)	101	463	-
Handedness						
Direction of handedness	Edinburgh handedness inventory	485	381 (257/124)	104	464	21
Degree of handedness	Box marking test	465	364 (239/125)	101	465	-
Questionnaires						
Behaviour & Health	DHBQ	490	375 (254/121)	115	451	39
Autistic traits Self-report	Autism-Spectrum Quotient	488	383 (258/125)	105	467	21
Autistic traits Maternal-report	Social Responsiveness Scale, short version	217 (from 77 families)	154	63	154	-
Pubertal development	Tanner	462	357 (242/115)	105	433	29
Behaviour during tests	Test observation form	278	219 (107/112)	59	278	-
Home situation†	Living with parents or independent	352	265 (179/86)	87	352	-
Acne†	Acne problems	347	262 (178/84)	85	347	-
Hormones						
testosterone	Saliva samples	452	345 (227/118)	107	427	25
cortisol	Saliva samples	454	347 (230/117)	107	427	27
DNA						
DNA buccal swabs		471	357 (235/122)	114	443	28
Dermatoglyphic data						
Dermatoglyphic data	Ridge count fingerprints	420	330 (217/113)	90	420	-
Cardiovascular function						
Systolic and diastolic blood pressure		459	358 (234/124)	101	459	-
Heart rate		459	358 (234/124)	101	459	-
Heart rate variability	VU-AMS	452	355 (231/124)	97	452	-

Note: N Total = total number of participating subjects; N twins = total number of participating twins (*in parentheses: number of twins in longitudinal study/number of new participating twins); N siblings = total number of participating siblings; N VU: number of subjects participating in laboratory protocol at the VU University; N home: Number of subjects who did not visit the VU laboratory, but were willing to collect data at home. †Data collected per mail after testing day.

Psychological test protocol

After lunch, the participants completed the psychological test protocol. A complete overview of all data that were collected is given in Table 3. A description of the tasks and measures used in this protocol is presented below.

Cognition

Wechsler IQ

All participants of 16 years of age or above completed 11 subtests of the Dutch version of the Wechsler Adult Intelligence Scale-Third edition (WAIS-III; Wechsler, 1997). Verbal and nonverbal intelligence scores were calculated as the mean subtest score on the 6 verbal, respectively the 5 nonverbal subtests. The participants younger than 16 years completed the Dutch version of the full Wechsler Intelligence Scale for Children-Third edition (WISC-III; Wechsler, 2002). Verbal and nonverbal IQ scores were measured as the standardized score on 5 verbal subtests, and 5 nonverbal subtests. Both in the WAIS-III and the WISC-III, the scores were standardised for the appropriate age group, based on a population sample of same-aged subjects in the Netherlands. Standardisation norms were the same across the sexes.

Stroop

In the Stroop colour word task (Stroop, 1935), the participants have to complete 3 cards, each of which encompasses 10 rows with 10 items. The participants are asked to name aloud the items on each card. The first card involves naming the words 'red', 'green', 'yellow', and 'blue', printed in black ink. The second card involves naming the colour of the ink in which small rectangles on the card are printed. The third card contains names of colours printed in incongruent colours. The participant is asked to name the colour of the ink in which the word is printed and to not read the word itself. The performance on each card is scored as the time it takes to complete it (in seconds) and the number of errors made (i.e. the number of incorrectly named items, or skipped items). The Stroop effect or Colour word interference effect is computed as the difference in time between performance on card 3 and card 2. The Stroop effect is thought to be a measure of inhibition, as the participants have to inhibit the tendency to produce a dominant or automatic response (i.e. the content of the word instead of the colour of the ink).

Verbal fluency

This test evaluates the spontaneous production of words starting with a certain letter (verbal fluency letters) or belonging to a certain semantic category (verbal fluency categories) within a limited amount of time. The participants completed 2 trials for both conditions, and were instructed to name as many words as possible in one minute starting with an R or a T (letter trials), or belonging to the category "animals" or "professions" (category trials). Within the letter trials, subjects were prohibited from saying proper nouns (e.g. Robert or Rotterdam) or saying the same word twice using a different ending (e.g. roast and roasted). The total number of correct words per trial was recorded.

CVLT

The California Verbal Learning Test (CVLT; Mulder et al., 1996) is a measure of verbal learning and memory. In this task, a list of 16 items, with four words from each of four categories (fruits; herbs and spices; clothing; tools) is presented. The list is presented five times, and the participant is instructed to recall as many words as possible from the list following each presentation. Subsequently, a second list consisting of 16 items is presented (the interference list). After hearing the interference list, the subject is asked to recall as many items from the original list (short delay free recall), and to recall as many items from each of the semantic categories (cued recall). After a time interval of 20 minutes (in which the participants completed an emotion recognition task and an inspection time task), long delay free recall of the items is assessed, following by an assessment of cued recall and recognition of the items. Performance on each trial was measured by the number of correctly recalled words, perseverations, and intrusions.

Reading the mind in the eyes

The Reading the mind in the eyes task (Baron-Cohen et al., 2001a) is an advanced theory of mind test and aims to assess the ability to "read" mental states of another person. The original English version of the test (Baron-Cohen et al., 2001a) was translated into Dutch using the backward translation procedure. The participant is presented with a series of 36 photographs of the eye-region of the face of different actors and actresses, and is asked to choose which of four words best describes what the person in the photograph is thinking or feeling. Test performance is scored as the total number of correct answers.

Π inspection time task

The version of the Π inspection time task used in this protocol was designed after Luciano and colleagues (Luciano et al., 2001) and is a measure of perceptual speed. The participants are presented with a Π figure, are told that the two legs of the figure represent worms, and are asked to identify which worm is the longest. The Π figure is only presented shortly, after which the worms burrow quickly into the ground (i.e. disappear from the screen in the form of two lightning bolts). After 5 correct respons-

es, the participant "caught enough worms to catch a fish", and a fish appeared in the bottom left corner of the screen. The test uses a staircase method in which the stimulus duration (the Π figure) is altered based on the participant's response. After four consecutive correct responses, the stimulus duration of the next trial is decreased. In case of an incorrect answer, the stimulus duration of the subsequent trial is increased. The step size of the decrease/increase is dependent on previous performance. Thus, after many reversals, the increases/decreases on subsequent trials become smaller and the stimulus duration converges on the subject's minimal inspection time. The protocol was stopped when the minimum inspection time was achieved, or when the maximum of 96 trials was reached.

n-back task

Working memory was assessed using a spatial variant of the n-back task (Jansma et al., 2000) designed after Gevins and Cutillo (1993). The original task was adapted to make it more attractive for young participants (Van Leeuwen et al., 2007). Participants were requested to look at an apple presented on a screen. The apple had four holes in which a caterpillar could appear. Participants were told to catch the caterpillar to prevent it from eating the apple. They were instructed to respond to the caterpillar by pushing one of four buttons, corresponding to the four holes in which the caterpillar could appear. Participants had to indicate where the caterpillar was one move back (1-back), two moves back (2-back), three moves back (3-back) or four moves back (4-back). Sessions were given in blocks of 20 trials. After each block the participants received feedback on the number of apples they had saved from the caterpillar (correct responses) and how many had been eaten (incorrect responses). The 1-back and 2-back conditions were administered once and for practice purposes only. The 3-back and 4-back conditions encompassed one practice block and three blocks in which performance was recorded. Performance on the task was scored as the total number of correct responses per condition, with a maximum score of 60.

Corsi block tapping task

The Corsi block tapping task (Corsi, 1974) is a test of spatial short term memory. In this task, nine white blocks are presented on a touch screen monitor. One by one, some of the blocks turn red, after which the whole screen turns blank. Next, the nine white blocks are shown again and the participant is instructed to tap the blocks that just turned red and to do so in the correct order. The test started with two practice trials, in which a series of two blocks turned red. The actual testing phase started with a series of two blocks. After five trials the difficulty level was raised by increasing the series with one block. The test was terminated if the participant responded incorrectly (i.e. tapped the wrong blocks, or tapped the blocks in the incorrect order) in three out of the five trials. Performance was recorded as the total number of correct trials.

Handedness

Edinburgh handedness inventory

Direction of handedness was assessed using 7 items from the Edinburgh Handedness Inventory (Oldfield, 1971). The 7 items were selected following the results of a confirmatory factor analysis by Dragovic (2004). The participants are asked to state their hand preference in 7 activities (writing; throwing; using scissors; tooth brushing; eating with a fork (without a knife); using a spoon; striking a match), and rate their preference in each activity on a 5-point Likert scale (strong preference left hand/ left hand/ indifferent/right hand/ strong preference right hand).

Box marking test

Degree of handedness was measured using an adaptation (Leask & Crow, 2001) of the handedness task described by Tapley and Bryden (1985). Hand skill was assessed by the number of boxes the participant could tick on a printed sheet in one minute. Each hand was tested separately, the right hand being tested first.

Questionnaires

Behavioural ratings and information about physical development were obtained as part of this study by questionnaires that were completed by the twins themselves, their mothers and by the research assistants. In addition, questionnaire information was available from the NTR longitudinal data collection in twins (Bartels et al., 2007).

Self report: DHBQ

All participants were asked to fill out the Dutch Health and Behaviour Questionnaire (DHBQ), an extensive questionnaire encompassing the Youth Self Report (Achenbach & Rescorla, 2001; Verhulst et al., 1997) to assess problem behaviour, and questions concerning pubertal status, physical health, grade in school, sports participation, leisure activities, peer and self smoking behaviour and drug use, eating problems, self esteem, life events, religiosity, happiness, life satisfaction, family situation and family functioning (Bartels et al., 2007).

Self report: Autism-Spectrum Quotient

The Autism-Spectrum Quotient (AQ) is a self-administered questionnaire developed to quantify autistic traits in individuals with normal intelligence (Baron-Cohen

et al., 2001b). The AQ consists of 50 items, selected from the domains of the "triad of impairment" in autism (impaired social skills, impaired communication, and restricted interests), and from demonstrated areas of cognitive abnormality in autism (e.g. lack of imagination and great attention to detail). Subjects rate to what extent they agree or disagree with the statements on a 4-point Likert scale, with answer categories "1 = definitely agree"; "2 = slightly agree"; "3 = slightly disagree" and "4 = definitely disagree". For items in which an "agree" response is characteristic for autism (24 out of the 50 items), the scoring is reversed. AQ scores are calculated as the sum scores, with a minimum AQ score (50) indicating no autistic traits, and a maximum score (200) indicating full endorsement of all autistic traits.

Mother-report: Social Responsiveness Scale – short version

The Social Responsiveness Scale (SRS: Constantino, 2002) is a parent or teacher report questionnaire designed to assess autistic symptoms. The original version of the SRS comprises 65 items. In this study, we asked the mothers of the participants to fill out the short version of the SRS, including 16 items. Items were selected on the basis of an amalgam of factors, including high factor loading on the primary factor analytic domain of the SRS (see Constantino et al., 2004), as well as representation of all aspects of the autistic syndrome, including social impairment, language impairment, and stereotypic behaviours/restricted range of interest. The short SRS was translated into Dutch using the backward translation procedure. As this questionnaire only became available in December 2006, data were collected in a subset of the entire twin family sample, and comprised 77 families.

Self-report: Tanner

Pubertal development of the twins and their siblings was assessed using an extended version of the self-report Tanner questionnaires (Marshall & Tanner, 1969; Marshall & Tanner, 1970). Girls were asked whether they had experienced their menarche ("no"/"yes", if "yes" the date), and to rate their stage of breast development (5 categories) and pubic hair development (6 categories). Boys were asked about their genital development (5 categories), pubic hair development (6 categories) and testes size (4 categories). The different stages of pubertal development were illustrated by photographs.

Research assistant-report: Test observation form

The Test Observation Form (McConaughy & Achenbach, 2004) is a questionnaire designed to assess the problem behaviour of the participant during the test procedure, and is filled out by the test administrator at the end of the testing day. The form is designed parallel to the Child Behavior Checklist (Achenbach & Rescorla, 2001; Verhulst et al., 1996) and the Youth Self Report (Achenbach & Rescorla, 2001; Verhulst et al., 1997) and comprises 125 items. The test administrator rates the behaviour of the participant on a 4-point scale, and rates 0 if there was "no occurrence" of the behaviour, 1 if the behaviour occurred "very slight or ambiguous", 2 if the behaviour occurred "definite but mild to moderate and ≤ 3 minutes", and 3 if the behaviour occurred "definite, severe, high frequency, or > 3 minutes". The Test observation form was included in the test protocol after the start of the project, behavioural ratings are available for 278 participants.

Mother-report: Living with parents/independent

After the project had finished, the mothers of the participants received a mailing with some additional questions and, if necessary, enquiring about missing data. Within this mailing, the mothers were asked to provide information on whether the children who participated in the study still lived with their parents, or had moved out. If they had moved out, the mothers were asked the date when the child moved out.

Mother-report: Acne problems

After the end of the project, mothers of the participants were asked to provide some additional information by mail on the children who participated in the study. The mother was asked whether the child had (had) acne during puberty, prior to possible medication (such as oral contraceptives). The mother was asked about acne problems on the face, on the back, and on the chest, and was asked to rate the problems as 0 "no or hardly any"; 1 "moderate" or 3 "severe" problems.

Hormone sample collection

Salivary cortisol and testosterone levels

Saliva collection devices were sent to the twin families by mail prior to the testing day and samples were collected at home. The participants were instructed to collect their samples on the same day as their siblings and to do so on two week days, to try to restrict the awakening time and time of sampling. Each participant was asked to write down the exact sampling time in a "saliva diary", and to note exceptional events interfering with the daily routine. Moreover, their awakening times on the sampling day and on the two days prior to the day of sampling were noted. Subjects were instructed not to brush their teeth and not to eat or drink in the 30 minutes preceding the saliva collection. Saliva samples were stored in a refrigerator at the twin family's home and brought to the laboratory on the day of testing. In the laboratory, samples were stored at -20°C until the immunoassay analyses.

For the assessment of cortisol levels, participants were requested to collect saliva at five time points on one day using the salivette, a polyester saliva collection device (Sarstedt AG & Co., Nümbrecht, Germany). The first sample was taken in the morning just before getting up, the second, third and fourth sample were taken respectively 15, 30 and 45 minutes after the first sample. The last sample was taken just before lunch. At the fifth time point, the participants were asked to collect an additional saliva sample by passive drool, for the analysis of testosterone levels. The participants were asked to follow this procedure twice in one week, resulting in 10 (2 x 5) cortisol samples and 2 testosterone samples.

DNA collection

DNA was collected from buccal swabs and extracted from blood samples. Blood samples were collected in twins and their siblings at the beginning of the testing day. Participants were instructed not to eat and drink before the blood samples were taken and were served food and drinks afterwards. DNA from buccal swabs was collected both in the twins, their siblings, and in the parents of the children. All twin family members were mailed a DNA sample collection kit prior to the day of testing. The kit contained a tube with 16 cotton buds, 4 tubes with collection buffer and a sampling protocol. Subjects were asked to take 2 series of mouth swabs on 2 days, by consecutively rubbing 4 cotton buds along the inside of the mouth. After rubbing the mouth swabs were placed in a tube with buffer. Participants were instructed not to eat, drink, or brush their teeth before the DNA collection and were asked to bring the DNA samples to the VU laboratory on the day of testing, or the return the package to the university by mail. The DNA collected from the buccal swabs was used to test the zygosity of the twins

Dermatoglyphic data

The complex ridge patterns on the finger tips (dermatoglyphics) are unique to humans and formed in the embryonic phase. Anything affecting the dermal ridge patterns must occur prior to the middle of the 2nd trimester of development, and it is thought that dermatoglyphics are informative for early disturbances in development (see e.g. Van Oel et al., 2001). In order to calculate the ridge counts, prints of all finger tips of the participants were taken using a scanner.

Cardiovascular function

At four time points during the testing day, systolic and diastolic blood pressure and heart rate was measured using a Spacelabs 90207 device (Redmont, Washington, USA). During the entire testing day, electrocardiogram and impedance cardiogram were recorded using the VU-AMS (De Geus et al., 1995; Willemsen et al., 1996). At

the beginning of the testing day, the cardiovascular monitoring device was attached to the participant's body by placing 4 electrodes on the chest, 2 electrodes on the back, and attaching the device around the waist of the participant using a belt. Every time when a new part of the test protocol began, the participant pressed the "event button" on the VU-AMS, so that each activity of the day could be labelled as such. By doing so, cardiovascular functioning could be measured per test condition. At the end of the day of testing the device was detached from the participant's body.

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APPENDIX II: DUTCH TRANSLATION OF THE AUTISM-SPECTRUM QUOTIENT

Vragenlijst naar Gedrag en Persoonlijkheid

Dit is een vragenlijst over jezelf. Alle informatie wordt strikt vertrouwelijk behandeld.
Sekse: ☐V ☐M
Geboortedatum: _____/_____/_____(dag/maand/jaar)
Datum vandaag: _____/_____/_____(dag/maand/jaar)

Deze lijst bestaat uit een aantal uitspraken waarmee je het eens of oneens kunt zijn.
Lees iedere uitspraak zorgvuldig en omcirkel dan het antwoord dat het meest op je van toepassing is.
Voor iedere uitspraak is een viertal antwoordmogelijkheden gegeven:

1 = geheel mee eens 2 = enigszins mee eens 3 = enigszins mee oneens 4 = geheel mee oneens

1.	Ik doe dingen liever met anderen dan alleen.	**1**	**2**	**3**	**4**	12.	Mij vallen vaak details op die anderen niet zien.	**1**	**2**	**3**	**4**
2.	Ik doe dingen het liefst steeds weer op dezelfde manier.	**1**	**2**	**3**	**4**	13.	Ik zou liever naar een bibliotheek gaan dan naar een feest.	**1**	**2**	**3**	**4**
3.	Als ik me iets probeer voor te stellen, kan ik me makkelijk een beeld voor de geest halen.	**1**	**2**	**3**	**4**	14.	Ik vind het gemakkelijk om verhalen te verzinnen.	**1**	**2**	**3**	**4**
4.	Ik word vaak zo door iets in beslag genomen, dat ik andere zaken uit het oog verlies.	**1**	**2**	**3**	**4**	15.	Ik voel me meer aangetrokken tot mensen dan tot dingen.	**1**	**2**	**3**	**4**
5.	Ik merk vaak geluidjes op die anderen niet opvallen.	**1**	**2**	**3**	**4**	16.	Ik neig ernaar zeer sterke interesses te hebben, en ik raak van streek als ik die niet kan naleven.	**1**	**2**	**3**	**4**
6.	Mijn aandacht wordt vaak getrokken door nummerplaten van auto's, of soortgelijke rijtjes.	**1**	**2**	**3**	**4**	17.	Ik geniet van praten over koetjes en kalfjes.	**1**	**2**	**3**	**4**
7.	Andere mensen zeggen me vaak dat het onbeleefd is wat ik heb gezegd, terwijl ik zelf denk beleefd te zijn.	**1**	**2**	**3**	**4**	18.	Als ik praat, is het voor anderen niet altijd gemakkelijk om er een woord tussen te krijgen.	**1**	**2**	**3**	**4**
8.	Als ik een verhaal lees, kan ik me gemakkelijk voorstellen hoe de personages eruit zouden kunnen zien.	**1**	**2**	**3**	**4**	19.	Ik word gefascineerd door getallen.	**1**	**2**	**3**	**4**
9.	Ik word gefascineerd door jaartallen en data.	**1**	**2**	**3**	**4**	20.	Als ik een verhaal lees, vind ik het moeilijk om achter de bedoelingen van de personages te komen.	**1**	**2**	**3**	**4**
10	In een groep mensen kan ik gemakkelijk verschillende gesprekken tegelijk volgen.	**1**	**2**	**3**	**4**	21.	Ik ben niet echt een liefhebber van het lezen van romans.	**1**	**2**	**3**	**4**
11.	Ik vind sociale situaties gemakkelijk.	**1**	**2**	**3**	**4**	22.	Ik vind het moeilijk om nieuwe vrienden te maken.	**1**	**2**	**3**	**4**

1 = geheel mee eens 2 = enigszins mee eens 3 = enigszins mee oneens 4 = geheel mee oneens

23.	Ik merk steeds patronen op in dingen die ik zie.	**1**	**2**	**3**	**4**	37.	Na een onderbreking kan ik heel snel terugschakelen naar waar ik mee bezig was.	**1**	**2**	**3**	**4**
24.	Ik zou liever naar het theater gaan dan naar een museum.	**1**	**2**	**3**	**4**	38.	Ik ben goed in praten over koetjes en kalfjes.	**1**	**2**	**3**	**4**
25.	Ik raak niet van streek als mijn dagelijkse routine wordt verstoord.	**1**	**2**	**3**	**4**	39.	Mensen vertellen me vaak dat ik maar door blijf gaan over hetzelfde onderwerp.	**1**	**2**	**3**	**4**
26.	Ik merk vaak dat ik niet weet hoe ik een conversatie gaande moet houden.	**1**	**2**	**3**	**4**	40.	Toen ik klein was, vond ik het leuk om 'doen-alsof'-spelletjes met andere kinderen te spelen.	**1**	**2**	**3**	**4**
27.	Ik vind het gemakkelijk om 'tussen de regels door te luisteren' als iemand tegen mij praat.	**1**	**2**	**3**	**4**	41.	Ik vind het leuk om informatie te verzamelen over bepaalde categorieën van dingen (bijv. automerken, vogel-, trein-, plantensoorten, etc.)	**1**	**2**	**3**	**4**
28.	Gewoonlijk concentreer ik me meer op het hele beeld dan op de kleine details.	**1**	**2**	**3**	**4**	42.	Ik vind het moeilijk om me voor te stellen hoe het zou zijn als ik iemand anders was.	**1**	**2**	**3**	**4**
29.	Ik ben niet erg goed in het onthouden van telefoonnummers.	**1**	**2**	**3**	**4**	43.	Ik vind het prettig om alle activiteiten, waaraan ik deelneem, zorgvuldig te plannen.	**1**	**2**	**3**	**4**
30.	Kleine veranderingen in situaties, of in hoe iemand eruit ziet, merk ik meestal niet op.	**1**	**2**	**3**	**4**	44.	Ik geniet van sociale gebeurtenissen.	**1**	**2**	**3**	**4**
31.	Ik kan merken wanneer iemand die naar me luistert, verveeld raakt.	**1**	**2**	**3**	**4**	45.	Ik vind het moeilijk om achter de bedoelingen van anderen te komen.	**1**	**2**	**3**	**4**
32.	Ik vind het gemakkelijk om meer dan één ding tegelijk te doen.	**1**	**2**	**3**	**4**	46.	Nieuwe situaties maken me angstig.	**1**	**2**	**3**	**4**
33.	Als ik telefoneer, ben ik er niet zeker van wanneer het mijn beurt is om iets te zeggen.	**1**	**2**	**3**	**4**	47.	Ik vind het leuk om nieuwe mensen te ontmoeten.	**1**	**2**	**3**	**4**
34.	Ik vind het leuk spontaan iets te ondernemen.	**1**	**2**	**3**	**4**	48.	Ik ben een goede diplomaat.	**1**	**2**	**3**	**4**
35.	Ik ben vaak de laatste die de clou van een grap begrijpt.	**1**	**2**	**3**	**4**	49.	Ik ben er niet erg goed in de geboortedata van anderen te onthouden.	**1**	**2**	**3**	**4**
36.	Ik vind het gemakkelijk om erachter te komen wat iemand denkt of voelt, alleen door naar zijn of haar gezicht te kijken.	**1**	**2**	**3**	**4**	50.	Ik vind het erg gemakkelijk om 'doen-alsof'-spelletjes met kinderen te spelen.	**1**	**2**	**3**	**4**

Dit is het einde van deze vragenlijst. Controleer alsjeblieft je antwoorden om zeker te zijn dat je het juiste antwoord hebt omcirkeld. Bedankt voor je medewerking!

APPENDIX III: INFORMATION BROCHURE FOR PARENTS AND CHILDREN, LONGITUDINAL SAMPLE

algemeen

Tweelingonderzoek is belangrijk voor het wetenschappelijk onderzoek binnen de geneeskunde en de psychologie. Met onderzoek bij tweelingen kunnen wetenschappers erachter komen in hoeverre verschillen in gedrag of gezondheid worden beïnvloed door verschillen in erfelijke aanleg (de genen) of door leefomgeving. Om goed onderzoek te kunnen doen, zijn vaak gegevens nodig van veel tweelingen. Daarom is in 1987 aan de Vrije Universiteit (VU) in Amsterdam het Nederlands Tweelingen Register (NTR) opgericht. Bij het NTR staan nu zo'n 30.000 jonge en volwassen tweelingen ingeschreven.

De meeste gegevens van deze tweelingen worden verkregen met vragenlijsten die door tweelingen zelf, door hun ouders of leerkracht worden ingevuld. Soms worden tweelingen en hun gezinsleden uitgenodigd om naar de VU te komen voor onderzoek. Sommige tweelingen doen al meer dan 10 jaar mee aan deze onderzoeken en zijn een aantal keren in Amsterdam geweest. Verschillende wetenschappers maken gebruik van deze gegevens om de invloed van erfelijke aanleg en omgeving op de ontwikkeling van de hersenen, groei, cognitieve vaardigheden, gedragsproblemen, en leefgewoonten te onderzoeken. De belangeloze medewerking van tweelingen en hun gezinsleden is hierbij van onschatbare waarde.

huidig onderzoek

Uw gezin behoort tot een unieke groep die al herhaaldelijk heeft meegedaan aan tweelingonderzoek. Dankzij uw medewerking is er inmiddels bekend hoe belangrijk erfelijke aanleg is bij bijvoorbeeld de ontwikkeling van attentie- en concentratieproblemen bij kinderen.

Graag zouden we in dit vervolgonderzoek kijken in hoeverre gedurende de middelbare schooljaren veranderingen in cognitieve vaardigheden, gedrag en lichamelijke kenmerken zijn ontstaan. We hopen met dit onderzoek meer inzicht te krijgen in de ontwikkeling van kinderen in Nederland.

Jullie hebben al eerder meegedaan aan onderzoek naar cognitieve vaardigheden, attentie, geheugen, hormonen en gedrag. Nieuw aan deze 5e meting is dat we ditmaal dieper willen ingaan op de biologische en lichamelijke ontwikkeling. Voor dit onderzoek werkt het NTR samen met de afdeling kindergeneeskunde van het *VU Medisch Centrum* (VUmc) in Amsterdam. In het VUmc wordt veel onderzoek gedaan naar de relatie tussen geboortegewicht en lengte en gezondheidseffecten op latere leeftijd. Onderzoek bij tweelingen en bij hun broers en zussen kan meer inzicht bieden in deze relatie, en helpen om afwijkingen in groei- en puberteitsontwikkeling beter te begrijpen. Omdat tweelingen vaak eerder worden geboren en lichter zijn dan niet-tweelingen, willen we ook hun broers en zussen vragen mee te doen aan dit onderzoek.

wie doen er mee?

In het huidige onderzoek worden uiteraard de tweelingen weer gevraagd mee te doen. Daarnaast bestaat nu voor het eerst de mogelijkheid om ook de broers en zussen (tussen de 12 en 20 jaar) van de tweelingen uit te nodigen om mee te doen. Zoals gebruikelijk wordt de ouders gevraagd om aanvullende informatie. Hier komen we later op terug.

tweelingen en hun broers/zussen

De vragenlijsten en een deel van de bepalingen kunnen thuis worden gedaan. Voor het resterende deel van het onderzoek worden de tweeling en eventuele broers en zussen (en als het mogelijk is één of twee van de ouders), gevraagd gezamenlijk een dag naar de VU te komen.

De dag op de VU bestaat uit twee onderdelen. In het eerste deel zal er in het VUmc een gesprek met een arts plaatsvinden en wordt een lichamelijk onderzoek gedaan. Daarna wordt bij de afdeling Biologische Psychologie van de VU een aantal tests afgenomen, die aandachtsprocessen, geheugen, leesvaardigheid en cognitie meten. Meer informatie over deze onderdelen vindt u hieronder.

vragenlijsten (thuis)

De tweelingen en hun eventuele broers en zusjes krijgen twee vragenlijsten opgestuurd. Een korte lijst over hun lichamelijke ontwikkeling (invullen duurt ongeveer 5 minuten) en een iets langere gedragsvragenlijst. Het invullen van de 2e lijst neemt ongeveer een half uur in beslag.

hormoononderzoek (thuis)

Net als 5 jaar geleden willen we aan de tweeling (en nu ook aan broers en zussen) vragen thuis speeksel te verzamelen door een aantal malen op een watje te kauwen en in een buisje te spugen. In het speeksel kunnen de stress- en geslachtshormoonconcentraties worden gemeten. Om het stresshormoon goed te kunnen meten, moet op de ochtend van de testdag ook urine worden verzameld in een potje.

DNA onderzoek (thuis)

DNA is de drager van ons erfelijk materiaal. Met behulp van het DNA kunnen we onderzoeken welke genen een rol spelen bij lichaamsgroei en -samenstelling en bij attentie en geheugen. Afname van DNA gebeurt door een monduitstrijkje, waarbij een wattenstaafje langs de binnenkant van de wang wordt geschraapt. Dit wattenstaafje moet in een speciaal reageerbuisje worden bewaard en moet worden meegenomen naar de VU. Omdat dit een groot, langlopend onderzoek betreft, vragen we toestemming om het DNA-materiaal op te slaan om in toekomstige analyses binnen het terrein van dit onderzoek te gebruiken. Deze opslag gebeurt onder een nummer en de gegevens worden volledig vertrouwelijk behandeld. Meer over privacy richtlijnen vindt u verderop in deze folder. Verder geldt voor alle DNA analyses dat de resultaten alleen op groepsniveau (onherkenbaar voor individuele personen) worden gerapporteerd. Deelnemers aan het DNA-onderzoek kunnen dus geen individuele uitslag krijgen.

lichamelijk onderzoek (VU)

De gezinsleden worden op de afgesproken dag samen rond 10.00 uur 's ochtends in het VU medisch centrum verwacht. Hier worden verschillende metingen gedaan om groei in kaart te brengen, zoals lichaamslengte, gewicht en taille- en heupomtrek. Ook wordt onderzocht in welke mate de kinderen in de puberteit zijn en wordt de bloeddruk gemeten. Om de hartslag, ademhaling en hartslagvariabiliteit te meten worden een aantal plakkers (elektrodes) op de huid aangebracht. Omdat deze maten voor een belangrijk deel erfelijk zijn, zullen, indien aanwezig, ook bij de ouders lengte, gewicht en bloeddruk worden gemeten.

Ten slotte worden tijdens de testdag op het VUmc éénmalig enkele buisjes bloed afgenomen. Hierin kunnen hormonen en lipiden zoals cholesterol worden gemeten, die niet in speeksel of urine kunnen worden bepaald. In verband met het cholesterol- en hormoononderzoek moeten de kinderen nuchter zijn. Na dit deel van het onderzoek wordt een maaltijd aangeboden. Bij meisjes die al menstrueren, dient de bloedafname ongeveer op dag 2 à 4 na de start van de menstruatie plaats te vinden of in geval van pilgebruik op dag 3 à 7 van de pilloze week. Indien dit moment niet samenvalt met het bezoek aan de VU, kan de bloedafname thuis plaatsvinden op het juiste tijdstip.

psychologisch onderzoek (VU)

Na het lichamelijk onderzoek krijgen de kinderen een aantal aandachts-, geheugen en intelligentietests te doen. De intelligentie wordt gemeten met de Wechsler Adult Intelligence Scale (WAIS). Deze test is de volwassenen versie van de test die 5 jaar geleden is gedaan. In totaal nemen de tests ongeveer 3 1/2 uur in beslag. Geheugen en aandachtstaken worden grotendeels met de computer afgenomen.

de ouders

Voor het bezoek aan de de VU, krijgen de ouders een vragenlijst toegestuurd, waarin gevraagd wordt naar de periode van zwangerschap en geboorte van de kinderen. Graag zouden we ook, als dit mogelijk is, de oude groeigegevens van de kinderen ontvangen (bijvoorbeeld uit het zogenaamde 'groene boekje' van het consultatiebureau).

Tevens zouden wij graag toestemming van de ouders krijgen om groeigegevens op te vragen bij consultatiebureau, schoolarts en/of huisarts. Ook zouden we de ouders graag willen betrekken bij het DNA onderzoek. In dit erfelijkheidsonderzoek is het belangrijk uit te vinden welke genen van de moeder en welke van de vader zijn geërfd. Daarom willen we ook de ouders vragen eenmalig mee te werken aan DNA onderzoek, door met een monduitstrijkje DNA te verzamelen en dit mee te nemen of op te sturen naar de VU.

verzekering

De opdrachtgever voor bovengenoemd wetenschappelijk onderzoek, het *VU medisch centrum*, heeft u verzekerd in verband met eventuele schade die u zou kunnen lijden als gevolg van uw deelname aan dit onderzoek. Deze verzekering dekt schade door dood of letsel die het gevolg is van deelname aan het onderzoek, en die zich gedurende de deelname aan het onderzoek openbaart, of binnen vier jaar na beëindiging van de deelname aan het onderzoek. De schade wordt geacht zich te hebben geopenbaard wanneer deze bij de verzekeraar is gemeld.

In geval van schade kunt u zich direct wenden tot de verzekeraar.
De verzekeraar van het onderzoek is:
Onderlinge Waarborgmaatschappij Centramed b.a.
Postbus 90504
2509 LM Den Haag
De verzekering biedt een maximum dekking van:
€ 450.000 per proefpersoon en
€ 3.500.000 voor het gehele onderzoek en
€ 5.000.000 per jaar voor alle onderzoeken van dezelfde opdrachtgever.

De dekking van specifieke schades en kosten is verder tot bepaalde bedragen beperkt. Zie voor verdere informatie hieromtrent het Besluit verplichte verzekering bij medisch-wetenschappelijk onderzoek met mensen op de website van de Centrale Commissie Mensgebonden Onderzoek: **www.ccmo.nl**

Voor deze verzekering gelden een aantal uitsluitingen. De verzekering dekt niet:
schade waarvan op grond van de aard van het onderzoek zeker of nagenoeg zeker was dat deze zich zou voordoen;
- schade aan de gezondheid die ook zou zijn ontstaan indien u niet aan het onderzoek had deelgenomen;
- schade die het gevolg is van het niet of niet volledig nakomen van aanwijzingen of instructies;
- schade aan nakomelingen, als gevolg van een nadelige inwerking van het onderzoek op u of uw nakomeling;
- bij onderzoek naar bestaande behandelmethoden: schade die het gevolg is van één van deze behandelmethoden;
- bij onderzoek naar de behandeling van specifieke gezondheidsproblemen: schade die het gevolg is van het niet verbeteren of van het verslechteren van deze gezondheidsproblemen.

Indien u schade heeft geleden door het onderzoek of het vermoeden daarvan heeft dient u zich met de onderzoeker dan wel met het bureau medische zaken van het ziekenhuis (tel. 020-4443555) in verbinding te stellen.

Indien bovengenoemde de bedragen de schade niet volledig dekken en aangetoond kan worden dat de uitvoering van het onderzoek onzorgvuldig is geweest dan kunt u hiernaast ook het ziekenhuis dat of de industrie die opdracht gegeven heeft tot het onderzoek of het ziekenhuis waar het onderzoek is uitgevoerd aansprakelijk stellen.

privacy

Alle gegevens die met tweelingonderzoek worden verzameld, worden vertrouwelijk behandeld. De gegevens worden geregistreerd en verwerkt onder een nummer en dus niet onder een naam of andere persoonlijke gegevens. Ook in publicaties zijn namen niet terug te vinden.

praktische informatie

U bent geheel vrij in het al dan niet deelnemen aan het onderzoek. We adviseren u voldoende tijd te nemen om erover na te denken of u wilt meedoen.

- U kunt altijd zonder opgaaf van redenen stoppen met deelname aan het onderzoek. Dit zal geen nadelige gevolgen hebben wanneer u voor andere behandelingen ons ziekenhuis bezoekt.
- Mochten er tijdens het onderzoek bevindingen zijn, waarvoor medisch handelen noodzakelijk wordt gevonden, dan wordt u hiervan uiteraard op de hoogte gesteld.
- De gehele testprocedure op de VU duurt van 10.00 uur tot ongeveer 16.00 uur.
- De reiskosten die gemaakt zijn, zullen worden vergoed.
- Het is de bedoeling dat de tweeling (plus eventuele broertjes en zusjes) samen op dezelfde dag langskomen om te worden getest.
- De belangrijkste resultaten van het onderzoek worden thuisgestuurd.

extra informatie

Het Nederlands Tweelingen Register heeft een website. Surf naar: **www.tweelingenregister.org** en lees meer over het NTR, het onderzoek en de uitkomsten.

Voor meer informatie over dit onderzoek kunt u contact opnemen met één van de uitvoerend onderzoekers:
Rosa Hoekstra (biologische psychologie - VU)
tel. 020-4448363 e-mail **ra.hoekstra@psy.vu.nl**

Indien u er prijs op stelt informatie over dit onderzoek in te winnen bij een arts die niet bij de uitvoering van het onderzoek betrokken is, dan is prof. dr. H.N. Lafeber, kinderarts VUmc, bereid uw vragen te beantwoorden. Hij is te bereiken op telefoonnummer 020-4440800. Wij hopen u binnenkort weer terug te zien op de VU en danken u voor uw medewerking.

APPENDIX IVA: INVITATION LETTER TO THE PARENTS FROM THE LONGITUDINAL SAMPLE

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Datum postmerk		020-5988832	informatiefolder; brief voor kinderen.
Ons kenmerk	**Uw kenmerk**	**Telefoon**	**E-mail**
NTR/ROS		020-5988363/ 820	ra.hoekstra@psy.vu.nl / gf.estourgie@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije Universiteit amsterdam

Geachte heer en mevrouw

U en uw tweeling doen al sinds de tweeling 5 jaar was mee aan onderzoek naar de erfelijke invloeden op ontwikkeling. Uw tweeling is nu (bijna) 18 jaar en we willen u graag vragen of u en uw kinderen binnenkort weer mee willen werken aan het vervolg van dit onderzoek. Ook willen we dit keer eventuele broers of zussen vragen om mee te doen.

De gang van zaken rond het onderzoek lijkt op die van de vorige keren: u krijgt een aantal vragenlijsten toegestuurd om thuis in te vullen. Tevens krijgt uw tweeling een aantal buisjes thuisgestuurd voor het verzamelen van speeksel, urine, en een monduitstrijkje. Daarnaast wordt de tweeling en hun broers/zussen gevraagd om een dag naar de VU te komen voor onderzoek. Dit keer zal als onderdeel van het onderzoek ook een gesprek plaatsvinden met een arts. Zij zal een algemeen lichamelijk onderzoek doen, en wat bloed afnemen. De arts is werkzaam bij de afdeling kindergeneeskunde van het VU Medisch Centrum. De samenwerking met deze afdeling heeft tot doel om, naast het gebruikelijke psychologisch onderzoek, ook meer te weten te komen over erfelijke aspecten van lichamelijke gezondheid, groei en puberteitsontwikkeling.

We willen uw kinderen (de tweeling en eventuele broers of zusjes) vragen om samen een dag naar de VU te komen. In de bijgesloten informatiefolder wordt in meer detail verteld wat het onderzoek inhoudt, wat het belang ervan is en wat deelname voor u en uw kinderen betekent. Bijgevoegd is ook een brief die u, indien u toestemt in deelname, aan uw kinderen kunt geven.

Uw deelname aan het onderzoek is erg waardevol. Wij hopen dat u en uw kinderen ook dit keer weer mee willen werken. Binnenkort nemen wij hierover telefonisch contact met u op. Mochten u of uw kinderen na het lezen van deze brief nog vragen hebben, dan kunt u ook zelf contact opnemen met de uitvoerend onderzoekers, Rosa Hoekstra (tel. 020-5988363) of Frederiek Estourgie-van Burk (tel. 020-5988820). Mocht u liever eerst met een onafhankelijke arts praten, dan kunt u bellen met prof. dr. H.N. Lafeber (tel. 020-4440800). Verdere informatie over tweelingenonderzoek is ook te vinden op onze website: www.tweelingenregister.org.

Met vriendelijke groet,

Prof. dr. D.I. Boomsma
Nederlands Tweelingen Register

Prof. dr. H.A. Delemarre-van de Waal
kinderarts-endocrinoloog

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX IVB: INVITATION LETTER TO THE TWINS FROM THE LONGITUDINAL SAMPLE

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Datum postmerk		020-5988832	informatiefolder
Ons kenmerk	**Uw kenmerk**	**Telefoon**	**E-mail**
NTR/ROS		020-5988363/820	ra.hoekstra@psy.vu.nl / gf.estourgie@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije Universiteit amsterdam

Beste

Sinds je 5e verjaardag heb je een aantal keren meegedaan aan onderzoek bij het Nederlands Tweelingen Register. Graag willen we je uitnodigen nu opnieuw mee te doen. Juist omdat je al zo vaak aan ons onderzoek hebt meegedaan, is je inzet enorm waardevol. We hopen dat je binnenkort weer naar de VU wilt komen.

Het onderzoek houdt in dat we je, net als de vorige keer, verzoeken om thuis een vragenlijst over gedrag en lichamelijke ontwikkeling in te vullen en wat speeksel te verzamelen. Dan volgt een bezoek aan de VU, waar een aantal tests worden gedaan die attentie, concentratie, geheugen en cognitieve vaardigheden in kaart brengen. Nieuw aan dit onderzoek is, dat er eenmalig een gesprek zal plaatsvinden met een arts, die vervolgens een algemeen lichamelijk onderzoek zal verrichten. Verder wordt wat bloed afgenomen, en word je gevraagd urine en een monduitstrijkje te verzamelen. Dit deel van het onderzoek wordt gedaan in samenwerking met de afdeling kindergeneeskunde van het VU Medisch Centrum in Amsterdam. De samenwerking heeft tot doel om, naast het gebruikelijke psychologisch onderzoek, ook meer te weten te komen over biologische en lichamelijke aspecten van groei en puberteitsontwikkeling.

Bij deze brief zit een informatiefolder waarin precies beschreven staat hoe het onderzoek er uit zal zien. Heb je na het lezen van deze folder nog vragen, dan kun je contact opnemen met de uitvoerend onderzoekers, Rosa Hoekstra (tel. 020-5988363) of Frederiek Estourgie-van Burk (tel. 020-5988820). Voor meer informatie over het tweelingenonderzoek op de VU kun je ook kijken op www.tweelingenregister.org.

Wij zouden het fantastisch vinden als je een dagje naar de Vrije Universiteit in Amsterdam zou willen komen om aan ons onderzoek mee te doen. Binnenkort zullen wij telefonisch contact met je ouders opnemen. Als je weer mee wilt doen dan kunnen we een dag en tijdstip afspreken waarop je naar de Vrije Universiteit komt.

Met vriendelijke groet,

Prof. dr. D.I. Boomsma
Nederlands Tweelingen Register

Prof. dr. H.A. Delemarre-van de Waal
kinderarts-endocrinoloog

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX IVc: Invitation Letter to the Siblings from the Longitudinal Sample

Nederlands Tweelingen Register (NTR)

Datum Datum postmerk	Uw brief van	Telefax 020-5988832	Bijlage(n) informatiefolder
Ons kenmerk NTR/ROS	Uw kenmerk	Telefoon 020-5988363/820	E-mail ra.hoekstra@psy.vu.nl / gf.estourgie@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije **Universiteit** ***amsterdam***

Aan de broer en/of zus van

Zoals je wellicht weet doen je tweelingbroers en/of zussen mee aan een onderzoek bij het Nederlands Tweelingen Register van de Vrije Universiteit in Amsterdam. Dit keer willen we voor een vervolgonderzoek ook een broer of zus van de tweeling uitnodigen mee te doen.

Het onderzoek waar we je nu voor uitnodigen lijkt veel op de onderzoeken waaraan je tweelingbroers/zussen, en misschien ook jijzelf, eerder hebben meegedaan. We willen je vragen om thuis een vragenlijst over gedrag en lichamelijke ontwikkeling in te vullen. Op de VU worden een aantal tests gedaan die attentie, concentratie, geheugen en cognitie meten. Er zal ook eenmalig een gesprek plaatsvinden met een arts, die vervolgens een algemeen lichamelijk onderzoek zal verrichten. Verder wordt wat bloed afgenomen, en willen we je vragen thuis speeksel, urine, en een monduitstrijkje te verzamelen.

Bij deze brief zit een informatiefolder waarin precies beschreven staat hoe het hele onderzoek er uit zal zien. Mocht je na het lezen van deze folder nog vragen hebben over het onderzoek, dan kun je contact opnemen met de uitvoerend onderzoekers, Rosa Hoekstra (tel. 020-5988363) of Frederiek Estourgie-van Burk (tel. 020-5988820). Voor meer informatie over het tweelingenonderzoek op de VU kun je ook kijken op www.tweelingenregister.org.

Wij zouden het fantastisch vinden als je samen met je tweelingbroers en/of zussen een dagje naar de Vrije Universiteit in Amsterdam zou willen komen om aan ons onderzoek mee te doen. Binnenkort zullen wij telefonisch contact met je ouders opnemen. Als je mee wilt doen dan kunnen we een dag en tijdstip afspreken waarop je naar de Vrije Universiteit komt.

Met vriendelijke groet,

Prof. dr. D.I. Boomsma
Nederlands Tweelingen Register

Prof. dr. H.A. Delemarre-van de Waal
kinderarts-endocrinoloog

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX V: Information Brochure for Parents and Children, Newly Recruited Sample

algemeen

Tweelingonderzoek is belangrijk voor het wetenschappelijk onderzoek binnen de geneeskunde en de psychologie. Met onderzoek bij tweelingen kunnen wetenschappers erachter komen in hoeverre verschillen in gedrag of gezondheid worden beïnvloed door verschillen in erfelijke aanleg (de genen) of door leefomgeving. Om goed onderzoek te kunnen doen, zijn vaak gegevens nodig van veel tweelingen. Daarom is in 1987 aan de Vrije Universiteit (VU) in Amsterdam het Nederlands Tweelingen Register (NTR) opgericht. Bij het NTR staan nu zo'n 30.000 jonge en volwassen tweelingen ingeschreven.

De meeste gegevens van deze tweelingen worden verkregen met vragenlijsten die door tweelingen zelf, door hun ouders of leerkracht worden ingevuld. Soms worden tweelingen en hun gezinsleden uitgenodigd om naar de VU te komen voor onderzoek. Sommige tweelingen doen al meer dan 10 jaar mee aan deze onderzoeken en zijn een aantal keren in Amsterdam geweest. Verschillende wetenschappers maken gebruik van deze gegevens om de invloed van erfelijke aanleg en omgeving op de ontwikkeling van de hersenen, groei, cognitieve vaardigheden, gedragsproblemen, en leefgewoonten te onderzoeken. De belangeloze medewerking van tweelingen en hun gezinsleden is hierbij van onschatbare waarde.

huidig onderzoek

Dankzij tweelingonderzoek is er inmiddels bekend hoe belangrijk erfelijke aanleg is bij bijvoorbeeld de ontwikkeling van attentie- en concentratieproblemen bij kinderen in de basisschoolleeftijd.

Graag zouden we in dit onderzoek kijken in hoeverre gedurende de middelbare schooljaren veranderingen in cognitieve vaardigheden, gedrag en lichamelijke kenmerken zijn ontstaan. We hopen met dit onderzoek meer inzicht te krijgen in de ontwikkeling van jongeren in Nederland.

Naast cognitieve vaardigheden en gedrag richten wij ons in dit onderzoek ook op de biologische en lichamelijke ontwikkeling. Hiervoor werkt het NTR samen met de afdeling kindergeneeskunde van het VU Medisch Centrum (VUmc) in Amsterdam. In het VUmc wordt veel onderzoek gedaan naar de relatie tussen geboortegewicht en lengte en gezondheidseffecten op latere leeftijd. Onderzoek bij tweelingen en bij hun broers en zussen kan meer inzicht bieden in deze relatie, en helpen om afwijkingen in groei- en puberteitsontwikkeling beter te begrijpen. Omdat tweelingen vaak eerder worden geboren en lichter zijn dan niet-tweelingen, willen we ook hun broers en zussen ook vragen mee te doen aan dit onderzoek.

wie doen er mee?

Voor deze studie zijn wij op zoek naar tweelingen van (bijna) 18 jaar. Daarnaast nodigen wij ook broers en zussen van de tweelingen uit om mee te doen. De ouders wordt gevraagd om aanvullende informatie te geven. Hier komen we later op terug.

tweelingen en hun broers/zussen

De vragenlijsten en een deel van de bepalingen kunnen thuis worden gedaan. Voor het resterende deel van het onderzoek worden de tweelingen en eventuele broers en zussen gevraagd gezamenlijk een dag naar de VU te komen.

De dag op de VU bestaat uit twee onderdelen. In het eerste deel zal er in het VUmc een gesprek met een arts plaatsvinden en wordt een lichamelijk onderzoek gedaan. Daarna wordt bij de afdeling Biologische Psychologie van de VU een aantal tests afgenomen, die aandachtsprocessen, geheugen, leesvaardigheid en cognitie meten. Meer informatie over deze onderdelen vindt u hieronder.

vragenlijsten (thuis)

De tweelingen en hun eventuele broers en zussen krijgen twee vragenlijsten opgestuurd. Een korte lijst over hun lichamelijke ontwikkeling (invullen duurt on-geveer 5 minuten), en een iets langere gedragsvragenlijst. Het invullen van de 2e lijst neemt ongeveer een half uur in beslag.

hormoononderzoek (thuis)

We willen aan de tweeling en eventuele broers en zussen vragen thuis speeksel te verzamelen door een aantal malen op een watje te kauwen en in een buisje te spugen. In het speeksel kunnen de stress- en geslachtshormoonconcentraties worden gemeten. Om het stresshormoon goed te kunnen meten, moet op de ochtend van de testdag ook urine worden verzameld in een potje.

DNA onderzoek (thuis)

DNA is de drager van ons erfelijk materiaal. Met behulp van het DNA kunnen we onderzoeken welke genen een rol spelen bij lichaamsgroei en -samenstelling en bij attentie en geheugen. Afname van DNA gebeurt door een monduitstrijkje, waarbij een wattenstaafje langs de binnenkant van de wang wordt geschraapt. Dit wattenstaafje moet in een speciaal reageerbuisje worden bewaard en moet worden meegenomen naar de VU. Omdat dit een groot, langlopend onderzoek is het belangrijk uit te vinden welke genen van de moeder en welke van de vader zijn geërfd. Daarom willen we ook de ouders vragen eenmalig mee te werken aan DNA onderzoek, door met een monduitstrijkje DNA te verzamelen en te gebruiken. Deze opslag gebeurt onder een nummer en de gegevens worden volledig vertrouwelijk behandeld. Meer over privacy richtlijnen vindt u ver-derop in deze folder. Verder geldt voor alle DNA analyses dat de resultaten alleen op groepsniveau (onherkenbaar voor individuele personen) worden gerapporteerd. Deelnemers aan het DNA-onderzoek kunnen dus geen individuele uitslag krijgen.

lichamelijk onderzoek (VUmc)

De gezinsleden worden op de afgesproken dag samen rond 10.00 uur 's ochtends in het VU medisch centrum verwacht. Hier worden verschillende metingen gedaan om groei in kaart te brengen, zoals lichaamslengte, gewicht en taille- en heupomtrek. Ook wordt onderzocht in welke mate de kinderen in de puberteit zijn en wordt de bloeddruk gemeten. Om de hartslag, ademhaling en hartslagvariabiliteit te meten worden een aantal plakkers (elektrodes) op de huid aangebracht. Omdat deze maten voor een belangrijk deel erfelijk zijn, zullen, indien aanwezig, ook bij de ouders lengte, gewicht en bloeddruk worden gemeten.

Ten slotte worden tijdens de testdag op het VUmc éénmalig enkele buisjes bloed afgenomen. Hierin kunnen hormonen en lipiden zoals cholesterol worden gemeten, die niet in speeksel of urine kunnen worden bepaald. In verband met het cholesterol- en hormoononderzoek moeten de kinderen nuchter zijn. Na dit deel van het onderzoek wordt een maaltijd aangeboden.

psychologisch onderzoek (VU)

Na het lichamelijk onderzoek krijgen de kinderen een aantal aandachts-, geheugen en intelligentietests te doen. De intelligentie wordt gemeten met de Wechsler Adult Intelligence Scale (WAIS). In totaal nemen de tests ongeveer 3 en half uur in beslag. Geheugen en aandachtstaken worden grotendeels met de computer afgenomen.

de ouders

Voor het bezoek aan de VU, krijgen de ouders een vragenlijst toegestuurd, waarin gevraagd wordt naar de periode van zwangerschap en geboorte van de kinderen. Graag zouden we ook, als dit mogelijk is, de oude groeigegevens van de kinderen ontvangen (bijvoorbeeld uit het zogenaamde *groene boekje* van het consultatiebureau).

Tevens zouden wij graag toestemming van de ouders krijgen om groeigegevens op te vragen bij consultatiebureau, de schoolarts en/of de huisarts. Ook zouden we de ouders graag betrekken bij het DNA onderzoek. In dit erfelijkheidsonderzoek is het belangrijk uit te vinden welke genen van de moeder en welke van de vader zijn geërfd. Daarom willen we ook de ouders vragen eenmalig mee te werken aan DNA onderzoek, door met een monduitstrijkje DNA te verzamelen en

dit mee te nemen of op te sturen naar de VU.

privacy

Alle gegevens die met tweelingonderzoek worden verzameld, worden vertrouwelijk behandeld. De gegevens worden geregistreerd en verwerkt onder een nummer en dus niet onder een naam of andere persoonlijke gegevens. Ook in publicaties zijn namen niet terug te vinden.

verzekering

De opdrachtgever voor bovengenoemd wetenschappelijk onderzoek, het *VU medisch centrum*, heeft u verzekerd in verband met eventuele schade die u zou kunnen lijden als gevolg van uw deelname aan dit onderzoek. Deze verzekering dekt schade door dood of letsel die het gevolg is van deelname aan het onderzoek, en die zich gedurende de deelname aan het onderzoek openbaart, of binnen vier jaar na beëindiging van de deelname aan het onderzoek. De schade wordt geacht zich te hebben geopenbaard wanneer deze bij de verzekeraar is gemeld.

In geval van schade kunt u zich direct wenden tot de verzekeraar.
De verzekeraar van het onderzoek is:
Onderlinge Waarborgmaatschappij Centramed b.a.
Postbus 90504
2509 LM Den Haag
De verzekering biedt een maximum dekking van:
€450.000 per proefpersoon en
€3.500.000 voor het gehele onderzoek en
€5.000.000 per jaar voor alle onderzoeken van dezelfde opdrachtgever.

De dekking van specifieke schades en kosten is verder tot bepaalde bedragen beperkt. Zie voor verdere informatie hieromtrent het Besluit verplichte verzekering bij medisch-wetenschappelijk onderzoek met mensen op de website van de Centrale Commissie Mensgebonden Onderzoek: **www.ccmo.nl**

Voor deze verzekering gelden een aantal uitsluitingen. De verzekering dekt niet: schade waarvan op grond van de aard van het onderzoek zeker of nagenoeg zeker was dat deze zich zou voordoen;

- schade aan de gezondheid die ook zou zijn ontstaan indien u niet aan het onderzoek had deelgenomen;
- schade die het gevolg is van het niet of niet volledig nakomen van aanwijzingen of instructies;
- schade aan nakomelingen, als gevolg van een nadelige inwerking van het onderzoek op u of uw nakomeling;
- bij onderzoek naar bestaande behandelmethoden: schade die het gevolg is van één van deze behandelmethoden;
- bij onderzoek naar de behandeling van specifieke gezondheidsproblemen: schade die het gevolg is van het niet verbeteren of van het verslechteren van deze gezondheidsproblemen.

Indien u schade heeft geleden door het onderzoek of het vermoeden daarvan heeft, dient u zich met de onderzoeker dan wel met het bureau medische zaken van het ziekenhuis (tel. 020-598.35.55) in verbinding te stellen.

Indien bovengenoemde de bedragen de schade niet volledig dekken en aangetoond kan worden dat de uitvoering van het onderzoek onzorgvuldig is geweest dan kunt u hiernaast ook het ziekenhuis dat of de industrie die opdracht gegeven heeft tot het onderzoek of het ziekenhuis waar het onderzoek is uitgevoerd aansprakelijk stellen.

praktische informatie

U bent geheel vrij in het al dan niet deelnemen aan het onderzoek. We advi-seren u voldoende tijd te nemen om erover na te denken of u wilt meedoen.

- U kunt altijd zonder opgaaf van redenen stoppen met deelname aan het onderzoek. Dit zal geen nadelige gevolgen hebben wanneer u voor andere behandelingen ons ziekenhuis bezoekt.
- Mochten er tijdens het onderzoek bevindingen zijn, waarvoor medisch handelen noodzakelijk wordt gevonden, dan wordt u hiervan uiteraard op de hoogte gesteld.
- De gehele testprocedure op de VU duurt van 10.00 uur tot ongeveer 16.00 uur. Gedurende de dag zorgen wij voor eten en drinken.
- De reiskosten die gemaakt zijn, zullen worden vergoed.
- Het is de bedoeling dat de tweeling (plus eventuele broertjes en zusjes) samen op dezelfde dag langskomen om te worden getest.
- De belangrijkste resultaten van het onderzoek worden thuisgestuurd.

extra informatie

Het Nederlands Tweelingen Register heeft een website. Surf naar: **www.tweelingenregister.org** en lees meer over het NTR, het onderzoek en de uitkomsten.

Voor meer informatie over dit onderzoek kunt u contact opnemen met de uitvoerend onderzoekers:
Rosa Hoekstra (biologische psychologie - VU)
tel. 020-598.83.63 e-mail **ra.hoekstra@psy.vu.nl**

Frederiek Estourgie-van Burk (kindergeneeskunde - VUmc)
tel. 020-598.88.20 e-mail **f.estourgie@vumc.nl**

Indien u er prijs op stelt informatie over dit onderzoek in te winnen bij een arts die niet bij de uitvoering van het onderzoek betrokken is, dan is prof. dr. H.N. Lafeber, kinderarts VUmc, bereid uw vragen te beantwoorden. Hij is te bereiken op telefoonnummer 020-598.08.00. Wij hopen u binnenkort weer terug te zien op de VU en danken u voor uw medewerking.

APPENDIX VIa: Invitation Letter to the Parents from the Newly Recruited Sample

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Datum postmerk		020-5988832	informatiefolder; brief voor kinderen.
Ons kenmerk	Uw kenmerk	Telefoon	E-mail
NTR/ROS		020-5988363/ 820	ra.hoekstra@psy.vu.nl / gf.estourgie@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije* Universiteit *amsterdam

Geachte heer en mevrouw

U staat ingeschreven bij het Nederlands Tweelingen Register (NTR) van de Vrije Universiteit (VU) in Amsterdam. Deelname van tweelingfamilies aan NTR-onderzoek is voor ons van grote waarde.

Graag vragen we u toestemming voor een onderzoek naar de erfelijkheid van lichamelijke en geestelijke ontwikkeling bij uw tweeling en hun eventuele broers of zussen. Hiervoor willen we ze uitnodigen een dag naar de VU te komen. Het onderzoek bestaat uit twee delen: Het eerste deel is onderzoek naar lichamelijke gezondheid, groei en puberteit en gebeurt in samenwerking met het VU ziekenhuis. Het tweede deel gaat over leer- en geheugenfunctie en cognitie.

Ook wordt uw kinderen verzocht een vragenlijst in te vullen en genetisch materiaal (DNA, verkregen met een monduitstrijkje), ochtendurine en speeksel af te staan. Ook aan uzelf zouden wij willen vragen een korte vragenlijst in te vullen en met een monduitstrijkje DNA te verzamelen.

Al het onderzoek wordt vertrouwelijk gedaan.

Bij deze brief treft u een informatiefolder en een uw brief voor uw kinderen aan. In de folder staat waar het onderzoek over gaat, wat het belang is en wat deelname voor u en uw kinderen inhoudt. Als u toestemming wilt geven voor het onderzoek, kunt de brief aan uw kinderen geven.

Binnenkort nemen wij contact met u op voor het beantwoorden van vragen. Als u mee wilt doen aan het onderzoek, maken we een afspraak. Mocht u of uw kinderen direct al vragen hebben, dan kunt u contact op nemen met Rosa Hoekstra (tel. 020-5988363) of Frederiek Estourgie-van Burk (tel. 020-5988820). Indien u liever eerst met een onafhankelijke arts wilt praten, kunt u bellen met prof. dr. H.N. Lafeber (tel. 020-4440800). Verdere informatie over is ook te vinden op www.tweelingenregister.org.

Met vriendelijke groet,

Prof. dr. D.I. Boomsma
Nederlands Tweelingen Register

Prof. dr. H.A. Delemarre-van de Waal
kinderarts-endocrinoloog

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX VIB: INVITATION LETTER TO THE TWINS FROM THE NEWLY RECRUITED SAMPLE

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Datum postmerk		020-5988832	informatiefolder
Ons kenmerk	**Uw kenmerk**	**Telefoon**	**E-mail**
NTR/ROS		020-5988363/820	ra.hoekstra@psy.vu.nl / gf.estourgie@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije Universiteit *amsterdam*

Beste

Zoals je misschien wel weet sta je ingeschreven bij het Nederlands Tweelingen Register (NTR). Deelname van tweelingfamilies aan NTR-onderzoek is voor ons van onschatbare waarde. Graag willen we jou en je broers en/of zussen uitnodigen mee te doen aan een groot familieonderzoek naar de erfelijkheid van fysieke en mentale ontwikkeling.

Hiervoor willen we je uitnodigen een dag naar Amsterdam te komen. Het onderzoek bestaat uit twee delen: Het eerste deel is onderzoek naar lichamelijke gezondheid, groei en puberteitsontwikkeling en gebeurt in samenwerking met het VU ziekenhuis. Het tweede deel gaat over leer- en geheugenfunctie en cognitie. Daarnaast willen we je vragen om thuis een vragenlijst in te vullen en genetisch materiaal (DNA, verkregen door middel van een monduitstrijkje), ochtendurine en speeksel af te staan.

Bij deze brief zit een informatiefolder waarin precies beschreven staat hoe het onderzoek er uit zal zien. Heb je na het lezen van deze folder nog vragen, dan kun je contact opnemen met één van de uitvoerend onderzoekers: Rosa Hoekstra (tel. 020-5988363) of Frederiek Estourgie-van Burk (tel. 020-5988820). Voor meer informatie over het tweelingenonderzoek op de VU kun je ook kijken op www.tweelingenregister.org.

Wij zouden het fantastisch vinden als je een dag naar Amsterdam zou willen komen om aan ons onderzoek mee te doen. Binnenkort zullen wij telefonisch contact met je ouders opnemen. Als je mee wilt doen, dan kunnen we een dag en tijdstip afspreken waarop je naar de Vrije Universiteit komt.

Met vriendelijke groet,

Prof. dr. D.I. Boomsma
Nederlands Tweelingen Register

Prof. dr. H.A. Delemarre-van de Waal
kinderarts-endocrinoloog

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX VIC: INVITATION LETTER TO THE SIBLINGS FROM THE NEWLY RECRUITED SAMPLE

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Datum postmerk		020-5988832	informatiefolder
Ons kenmerk	**Uw kenmerk**	**Telefoon**	**E-mail**
NTR/ROS		020-5988363/820	ra.hoekstra@psy.vu.nl / gf.estourgie@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije Universiteit *amsterdam*

Aan de broer en/of zus van

Zoals je misschien wel weet staan je tweelingbroers en/of zussen ingeschreven bij het Nederlands Tweelingen Register (NTR). Deelname van tweelingfamilies aan NTR-onderzoek is voor ons van onschatbare waarde. Graag willen we jou en je tweelingbroers en/of zussen uitnodigen mee te doen aan een groot familieonderzoek naar de erfelijkheid van fysieke en mentale ontwikkeling.

Hiervoor willen we je uitnodigen een dag naar Amsterdam te komen. Het onderzoek bestaat uit twee delen: Het eerste deel is onderzoek naar lichamelijke gezondheid, groei en puberteitsontwikkeling en gebeurt in samenwerking met het VU ziekenhuis. Het tweede deel gaat over leer- en geheugenfunctie en cognitie. Daarnaast willen we je vragen om thuis een vragenlijst in te vullen en genetisch materiaal (DNA, verkregen door middel van een monduitstrijkje), ochtendurine en speeksel af te staan.

Bij deze brief zit een informatiefolder waarin precies beschreven staat hoe het onderzoek er uit zal zien. Heb je na het lezen van deze folder nog vragen, dan kun je contact opnemen met één van de uitvoerend onderzoekers: Rosa Hoekstra (tel. 020-5988363) of Frederiek Estourgie-van Burk (tel. 020-5988820). Voor meer informatie over het tweelingenonderzoek op de VU kun je ook kijken op www.tweelingenregister.org.

Wij zouden het fantastisch vinden als je een dag naar Amsterdam zou willen komen om aan ons onderzoek mee te doen. Binnenkort zullen wij telefonisch contact met je ouders opnemen. Als je mee wilt doen, dan kunnen we een dag en tijdstip afspreken waarop je naar de Vrije Universiteit komt.

Met vriendelijke groet,

Prof. dr. D.I. Boomsma
Nederlands Tweelingen Register

Prof. dr. H.A. Delemarre-van de Waal
kinderarts-endocrinoloog

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX VIIA: CONFIRMATION LETTER FOR THE TESTING DAY, TO THE FATHERS

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Datum postmerk		020-5988832	instructie; buizen met wattenstaafjes; verklaring; wegwijzer; antwoordenvelop.
Ons kenmerk	Uw kenmerk	Telefoon	E-mail
NTR/ROS/vader		020-5988363	ra.hoekstra@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije **Universiteit** ***amsterdam***

Geachte heer

Zoals met u aan de telefoon afgesproken is, ontvangt u hierbij een schriftelijke bevestiging van de afspraak voor de testdag op de Vrije Universiteit (VU). Uw tweeling (en hun broers en zusjes) worden op dag om 10 uur verwacht in het polikliniekgebouw van het VU medisch centrum (ingang De Boelelaan 1118) op de polikliniek kindergeneeskunde bij receptie L. Bij deze brief is een routebeschrijving gevoegd. Mochten uw kinderen onverhoopt verhinderd zijn, wilt u dan zo vriendelijk zijn om zo snel mogelijk contact met ons op te nemen? Ons telefoonnummer staat onderaan deze brief vermeld.

Bij deze brief ontvangt u:

- een instructie voor het nemen van een monduitstrijkje;
- een dikke buis met 16 wattenstaafjes en 4 dunne buisjes met vloeistof, voor het nemen van het monduitstrijkje;
- een verklaring van toestemming voor deelname aan dit onderzoek;
- een wegwijzer;
- antwoordenvelop.

Het is de bedoeling dat u de instructie voor het nemen van het monduitstrijkje eerst goed doorleest. Daarin wordt uitgelegd hoe u een monduitstrijkje kunt nemen en wat de belangrijke dingen zijn waar u op moet letten. De buisjes met de wattenstaafjes kunnen u of uw kinderen op de testdag meenemen naar de VU, of aan ons opsturen in bijgaande antwoordenvelop.

Uw kinderen ontvangen ieder een eigen brief. We willen u wel vragen de verklaring voor toestemming aan het onderzoek van uw minderjarige kinderen mede te ondertekenen. Deze verklaring zit bij de brief aan uw kind. Mochten u of uw kinderen na het lezen van deze brief nog vragen hebben over dit onderzoek, dan kunt u contact opnemen met één van de uitvoerend onderzoekers, Rosa Hoekstra (tel. 020-5988363).

Graag willen wij vragen of de kinderen i.v.m. het bloedonderzoek nuchter kunnen komen naar de testdag (niet eten en drinken ‘s ochtends). Tijdens het onderzoek zorgen wij voor eten en drinken, ook worden alle reiskosten vergoed. Bewaar daarvoor eventuele bonnen en bus- of treinkaartjes. Wanneer u met de auto komt verzoeken we u te parkeren op het parkeerterrein Gustav Mahlerlaan (achter de slagbomen), naast de polikliniek (zie wegwijzer). Bij binnenkomst kunt u een parkeerkaartje trekken, bij het weggaan zorgen wij voor een uitrijpas.

Hartelijk dank voor uw medewerking,
met vriendelijke groet,

mw. drs. Rosa Hoekstra
onderzoeker NTR

mw. drs. Frederiek Estourgie - van Burk
arts-onderzoeker VUmc

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX VIIB: CONFIRMATION LETTER FOR THE TESTING DAY, TO THE MOTHERS

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Datum postmerk		020-5988832	vragenlijst; instructie; buizen met wattenstaafjes; verklaring; wegwijzer; antwoordenvelop.
Ons kenmerk	Uw kenmerk	Telefoon	E-mail
NTR/ROS/moeder		020-5988363	ra.hoekstra@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije **Universiteit** ***amsterdam***

Geachte mevrouw

Zoals met u aan de telefoon afgesproken is, ontvangt u hierbij een schriftelijke bevestiging van de afspraak voor de testdag op de Vrije Universiteit (VU). Uw tweeling (en hun broers en zusjes) worden op dag om 10 uur verwacht in het polikliniekgebouw van het VU medisch centrum (ingang De Boelelaan 1118) op de polikliniek kindergeneeskunde bij receptie L. Bij deze brief is een routebeschrijving gevoegd. Mochten uw kinderen onverhoopt verhinderd zijn, wilt u dan zo vriendelijk zijn om zo snel mogelijk contact met ons op te nemen? Ons telefoonnummer staat onderaan deze brief vermeld.

Bij deze brief ontvangt u:

- twee vragenlijsten, één over uw zwangerschappen en één korte vragenlijst over het gedrag van uw kinderen;
- een instructie voor het nemen van een monduitstrijkje;
- een dikke buis met 16 wattenstaafjes en 4 dunne buisjes met vloeistof, voor het nemen van het monduitstrijkje;
- een verklaring van toestemming voor deelname aan dit onderzoek;
- een wegwijzer;
- antwoordenvelop.

Het is de bedoeling dat u de instructie voor het nemen van het monduitstrijkje eerst goed doorleest. Daarin wordt uitgelegd hoe u een monduitstrijkje kunt nemen en wat de belangrijke dingen zijn waar u op moet letten. De buisjes met de wattenstaafjes en de vragenlijst kunnen u of uw kinderen op de testdag meenemen naar de VU, of aan ons opsturen in bijgaande antwoordenvelop.

Uw kinderen ontvangen ieder een eigen brief. We willen u wel vragen de verklaring voor toestemming aan het onderzoek van uw minderjarige kinderen mede te ondertekenen. Deze verklaring zit bij de brief aan uw kind. Mochten u of uw kinderen na het lezen van deze brief nog vragen hebben over dit onderzoek, dan kunt u contact opnemen met één van de uitvoerend onderzoekers, Rosa Hoekstra (tel. 020-5988363).

Graag willen wij vragen of de kinderen i.v.m. het bloedonderzoek nuchter kunnen komen naar de testdag (niet eten en drinken ‘s ochtends). Tijdens het onderzoek zorgen wij voor eten en drinken, ook worden alle reiskosten vergoed. Bewaar daarvoor eventuele bonnen en bus- of treinkaartjes. Wanneer u met de auto komt verzoeken we u te parkeren op het parkeerterrein Gustav Mahlerlaan (achter de slagbomen), naast de polikliniek (zie wegwijzer). Bij binnenkomst kunt u een parkeerkaartje trekken, bij het weggaan zorgen wij voor een uitrijpas.

Hartelijk dank voor uw medewerking,
met vriendelijke groet,

mw. drs. Rosa Hoekstra
onderzoeker NTR

mw. drs. Frederiek Estourgie - van Burk
arts-onderzoeker VUmc

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX VIIc: Confirmation Letter for the Testing Day, to the Twins

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Datum postmerk		020-5988832	zie hieronder
Ons kenmerk	Uw kenmerk	Telefoon	E-mail
NTR/ROS/twin		020-5988363	ra.hoekstra@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije Universiteit *amsterdam*

Beste

Zoals afgesproken ontvang je hierbij een schriftelijke bevestiging van de afspraak voor de testdag op de Vrije Universiteit (VU). Je wordt op dag om 10 uur verwacht in het polikliniekgebouw van het VU medisch centrum (ingang De Boelelaan 1118) op de polikliniek kindergeneeskunde bij receptie L. Bij de brief aan je ouders is een routebeschrijving gevoegd. Mocht je onverhoopt niet kunnen komen, zou je dan zo snel mogelijk contact met ons kunnen opnemen? Ons telefoonnummer staat onderaan deze brief vermeld.

Bij deze brief ontvang je:

- twee vragenlijsten, één over lichamelijke ontwikkeling en één gedragsvragenlijst, beide door jou zelf in te vullen + een antwoordenvelop;
- een instructie en tijdschema voor het verzamelen van speeksel en urine;
- 10 plastic buisjes met een watje erin en 2 buisjes zonder watje, voor het nemen van de speekselmonsters;
- 1 plastic potje voor het verzamelen van de ochtendurine;
- een instructie voor het nemen van een monduitstrijkje;
- een dikke buis met 16 wattenstaafjes en 4 dunne buisjes met vloeistof, voor het nemen van het monduitstrijkje;
- een verklaring van toestemming voor deelname aan dit onderzoek;
- een beschrijving van het dagprogramma op de VU, met een checklist.

Het is de bedoeling dat je de instructies voor het verzamelen van het speeksel en urine, en voor het doen van het monduitstrijkje eerst goed doorleest. Daarin wordt uitgelegd hoe de verzameling in zijn werk gaat en wat de belangrijke dingen zijn waar je op moet letten. De buisjes met speeksel, de buisjes met wattenstaafjes, het potje ochtendurine en de ingevulde vragenlijsten kun je op de testdag meenemen naar de VU. Als je de vragenlijsten liever naar ons opstuurt kan dat ook, in bijgaande antwoordenvelop. Graag willen wij je vragen nuchter te komen naar de testdag (niet eten en drinken ‘s ochtends) i.v.m. het bloedonderzoek. Tijdens het onderzoek zorgen wij voor eten en drinken.

Je ouders hebben een eigen brief ontvangen. Als je minderjarig bent (jonger dan 18) dienen ook je ouders de verklaring voor toestemming aan het onderzoek te ondertekenen. Mocht je na het lezen van deze brief nog vragen hebben over het onderzoek, dan kun je contact opnemen met één van de onderzoekers, Rosa Hoekstra (tel. 020-5988363).

Hartelijk bedankt voor je medewerking en tot op de testdag op de VU!
Met vriendelijke groet,

mw. drs. Rosa Hoekstra
onderzoeker NTR

mw. drs. Frederiek Estourgie - van Burk
arts-onderzoeker VUmc

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX VIId: Confirmation Letter for the Testing Day, to the Siblings

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
Datum postmerk		020-5988832	zie hieronder
Ons kenmerk	Uw kenmerk	Telefoon	E-mail
NTR/ROS/sib		020-5988363	ra.hoekstra@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije Universiteit *amsterdam*

Beste

Zoals afgesproken ontvang je hierbij een schriftelijke bevestiging van de afspraak voor de testdag op de Vrije Universiteit (VU). Je wordt op dag om 10 uur verwacht in het polikliniekgebouw van het VU medisch centrum (ingang De Boelelaan 1118) op de polikliniek kindergeneeskunde bij receptie L. Bij de brief aan je ouders is een routebeschrijving gevoegd. Mocht je onverhoopt niet kunnen komen, zou je dan zo snel mogelijk contact met ons kunnen opnemen? Ons telefoonnummer staat onderaan deze brief vermeld.

Bij deze brief ontvang je:

- twee vragenlijsten, één over lichamelijke ontwikkeling en één gedragsvragenlijst, beide door jou zelf in te vullen + een antwoordenvelop;
- een instructie en tijdschema voor het verzamelen van speeksel en urine;
- 10 plastic buisjes met een watje erin en 2 buisjes zonder watje, voor het nemen van de speekselmonsters;
- 1 plastic potje voor het verzamelen van de ochtendurine;
- een instructie voor het nemen van een monduitstrijkje;
- een dikke buis met 16 wattenstaafjes en 4 dunne buisjes met vloeistof, voor het nemen van het monduitstrijkje;
- een verklaring van toestemming voor deelname aan dit onderzoek;
- een beschrijving van het dagprogramma op de VU, met een checklist;
- een registratiekaart voor het NTR.

Het is de bedoeling dat je de instructies voor het verzamelen van het speeksel en urine, en voor het doen van het monduitstrijkje eerst goed doorleest. Daarin wordt uitgelegd hoe de verzameling in zijn werk gaat en wat de belangrijke dingen zijn waar je op moet letten. Zou je ook de registratiekaart voor het NTR willen invullen? De registratiekaart, de buisjes met speeksel, de buisjes met wattenstaafjes, het potje ochtendurine en de ingevulde vragenlijsten kun je op de testdag meenemen naar de VU. Als je de vragenlijsten liever naar ons opstuurt kan dat ook, in bijgaande antwoordenvelop. Graag willen wij je vragen nuchter te komen naar de testdag (niet eten en drinken ‘s ochtends) i.v.m. het bloedonderzoek. Tijdens het onderzoek zorgen wij voor eten en drinken.

Je ouders hebben een eigen brief ontvangen. Als je minderjarig bent (jonger dan 18) dienen ook je ouders de verklaring voor toestemming aan het onderzoek te ondertekenen. Mocht je na het lezen van deze brief nog vragen hebben over het onderzoek, dan kun je contact opnemen met één van de onderzoekers, Rosa Hoekstra (tel. 020-5988363).

Hartelijk bedankt voor je medewerking en tot op de testdag op de VU!
Met vriendelijke groet,

mw. drs. Rosa Hoekstra
onderzoeker NTR

mw. drs. Frederiek Estourgie - van Burk
arts-onderzoeker VUmc

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

APPENDIX VIIIA: INFORMED CONSENT PARENTS

FAMILIEONDERZOEK NAAR DE ERFELIJKHEID VAN FYSIEKE EN MENTALE ONTWIKKELING

VERKLARING VAN TOESTEMMING NA KENNISNEMING

VOOR DE OUDER

Wilt u hieronder tekenen en daarmee het volgende verklaren:

1) De onderzoeker heeft mij volledig ingelicht over de aard en het doel van het "familieonderzoek naar de erfelijkheid van fysieke en mentale ontwikkeling" en ik ben op de hoogte van de onderzoeksmethoden en procedures.
2) Ik heb de informatie over dit onderzoek, die in de folder en brief worden gegeven, begrepen.
3) Ik heb de gelegenheid gehad vragen te stellen over dit onderzoek.
4) Ik begrijp dat ik te allen tijde de medewerking aan dit onderzoek mag afbreken zonder dat dit ongenoegen zal geven.

5) Ik heb toegestemd om deel te nemen aan de volgende onderzoeken:

Toestemming voor:		
* deelname aan het vragenlijstonderzoek	0 ja	0 nee
* deelname aan het DNA-onderzoek en de opslag van het erfelijk materiaal	0 ja	0 nee
* opvragen van de geboorte- en groeigegevens van mijn kinderen en het inzien van het Groene Boekje	0 ja	0 nee

6) Geeft u NTR toestemming uw gegevens te vergelijken met gegevens uit registratiesystemen? 0 ja 0 nee

Datum:

Naam betrokkene:

Onderzoekers:

Drs. R.A. Hoekstra
Drs. F. Estourgie-van Burk

Handtekening betrokkene:

Handtekening onderzoeker:

APPENDIX VIIIB: INFORMED CONSENT TWINS

FAMILIEONDERZOEK NAAR DE ERFELIJKHEID VAN FYSIEKE EN MENTALE ONTWIKKELING

VERKLARING VAN TOESTEMMING NA KENNISNEMING

VOOR DE OUDSTE VAN DE TWEELING

Wil je hieronder tekenen en daarmee het volgende verklaren:

1) De onderzoeker heeft mij volledig ingelicht over de aard en het doel van het "familieonderzoek naar de erfelijkheid van fysieke en mentale ontwikkeling" en ik ben op de hoogte van de onderzoeksmethoden en procedures.
2) Ik heb de informatie over dit onderzoek, die in de folder en brief worden gegeven, begrepen.
3) Ik heb de gelegenheid gehad vragen te stellen over dit onderzoek.
4) Ik begrijp dat ik te allen tijde de medewerking aan dit onderzoek mag afbreken zonder dat dit ongenoegen zal geven.
5) Ik heb toegestemd om deel te nemen aan de volgende onderzoeken:

Toestemming voor deelname aan		
* het vragenlijstonderzoek	0 ja	0 nee
* het hormoononderzoek	0 ja	0 nee
* het lichamelijk onderzoek	0 ja	0 nee
* de intelligentie- en aandachtstests	0 ja	0 nee
* het DNA-onderzoek en de opslag van het erfelijk materiaal	0 ja	0 nee

Datum:

Naam betrokkene:

Onderzoekers:

Drs. R.A. Hoekstra
Drs. F. Estourgie-van Burk

Handtekening betrokkene:

Handtekening onderzoeker:

Handtekening 1e ouder/verzorger:

Handtekening 2e ouder/verzorger:

APPENDIX VIIIc: INFORMED CONSENT SIBLINGS

FAMILIEONDERZOEK NAAR DE ERFELIJKHEID VAN FYSIEKE EN MENTALE ONTWIKKELING

VERKLARING VAN TOESTEMMING NA KENNISNEMING

VOOR DE BROER OF ZUS VAN EEN TWEELING

Wil je hieronder tekenen en daarmee het volgende verklaren:

1) De onderzoeker heeft mij volledig ingelicht over de aard en het doel van het "familieonderzoek naar de erfelijkheid van fysieke en mentale ontwikkeling" en ik ben op de hoogte van de onderzoeksmethoden en procedures.
2) Ik heb de informatie over dit onderzoek, die in de folder en brief worden gegeven, begrepen.
3) Ik heb de gelegenheid gehad vragen te stellen over dit onderzoek.
4) Ik begrijp dat ik te allen tijde de medewerking aan dit onderzoek mag afbreken zonder dat dit ongenoegen zal geven.
5) Ik heb toegestemd om deel te nemen aan de volgende onderzoeken:

Toestemming voor deelname aan		
* het vragenlijstonderzoek	0 ja	0 nee
* het hormoononderzoek	0 ja	0 nee
* het lichamelijk onderzoek	0 ja	0 nee
* de intelligentie- en aandachtstests	0 ja	0 nee
* het DNA-onderzoek en de opslag van het erfelijk materiaal	0 ja	0 nee

6) Ik geef toestemming tot het opnemen van mijn gegevens in het Nederlands Tweelingen Register als broer of zus van een tweeling. Inschrijving verplicht mij niet tot deelname aan verdere onderzoeken.

0 ja[§] 0 nee

[§]Zou je het NTR registratieformulier willen invullen?

Datum:

Naam betrokkene:

Handtekening betrokkene:

Onderzoekers:

Drs. R.A. Hoekstra
Drs. F. Estourgie-van Burk

Handtekening onderzoeker:

Alleen indien betrokkene jonger is dan 18 jaar:
Handtekening 1e ouder/verzorger:

Handtekening 2e ouder/verzorger:

List of Publications

Articles

Baron-Cohen, S., **Hoekstra, R.A.**, Knickmeyer, R., Wheelwright, S. (2006). The Autism-Spectrum Quotient (AQ) - Adolescent version. *Journal of Autism and Developmental Disorders, 36*, 343-50.

Estourgie–van Burk, G.F., Bartels, M., **Hoekstra, R.A.**, Polderman, T.J.C, Delemarre–van de Waal, H.A., Boomsma, D.I. The cognitive cost of catch-up growth. *Submitted.*

Hoekstra, R.A., Bartels, M., Boomsma, D.I. (2006). Heritability of testosterone levels in 12-year-old twins and its relation to pubertal development. *Twin Research and Human Genetics, 9*, 558-65.

Hoekstra, R.A., Bartels, M., Boomsma, D.I. Longitudinal genetic study of verbal and nonverbal IQ from early childhood to young adulthood. *Learning and Individual Differences, 17,* 97-114.

Hoekstra, R.A., Bartels, M., Cath, D.C., Boomsma, D.I. Factor structure of the broader autism phenotype and its diagnostic validity: a study using the Dutch translation of the Autism-Spectrum Quotient (AQ). *Under revision.*

Hoekstra, R.A., Bartels, M., Hudziak, J.J., Van Beijsterveldt, C.E.M., Boomsma, D.I. Genetic and environmental influences on the stability of withdrawn behavior in children: A longitudinal, multi-informant twin study. *Under revision.*

Hoekstra, R.A., Bartels, M., Hudziak, J.J., Van Beijsterveldt, C.E.M., Boomsma, D.I. Genetic and environmental covariation between autistic traits and behavioral problems. *Submitted.*

Hoekstra, R.A., Bartels, M., Van Leeuwen, M., Boomsma, D.I. Genetic architecture of verbal abilities in children and adolescents. *Under revision.*

Hoekstra, R.A., Bartels, M., Verweij, C.J.H., Boomsma, D.I. (2007). Heritability of autistic traits in the general population. *Archives of Pediatric & Adolescent Medicine, 161*, 372-7.

Knickmeyer, R., Wheelwright, S., **Hoekstra, R.A.**, Baron-Cohen, S. (2006). Age of menarche in females with Autism Spectrum Conditions. *Developmental Medicine and Child Neurology, 48*, 1007-8.

Ligthart, L., Bartels, M., **Hoekstra, R.A.**, Hudziak, J.J., Boomsma, D.I. (2005). Genetic contributions to subtypes of aggression. *Twin Research and Human Genetics*, *8*, 483-91.

Van Leeuwen, M., Van den Berg, S.M., **Hoekstra, R.A.**, Boomsma, D.I. (2007) Endophenotypes for intelligence in children and adolescents. *Intelligence, 35*, 369-80.

Published Abstracts

Boomsma, D.I., Bartels, M., van Beijsterveldt, C.E.M., Luciano, M., de Geus, E.J.C., Posthuma, D., Hottenga, J.J., **Hoekstra, R.A.**, Estourgie – van Burk, G.F., Delemarre – van de Waal, H.A., Martin, N.G. Heritability of head size in Dutch and Australian twin families at ages 5 – 50 years. *Behavior Genetics, in press.*

Hoekstra, R.A., Bartels, M., Boomsma, D.I. (2005). Heritability of testosterone levels in 12-year-old twins. *Twin Research and Human Genetics, 8,* p 418.

Hoekstra, R.A., Bartels, M., Estourgie – van Burk, G.F., Verweij, C.J.H., Boomsma, D.I. Genetic and environmental influences on autistic traits and its association with peer interaction: a general population twin study. *Behavior Genetics, in press.*

Hoekstra, R.A., Bartels, M., Hudziak, J.J., van Beijsterveldt, C.E.M., Boomsma, D.I. (2005). Genetic and environmental mechanisms underlying stability in childhood withdrawn behavior. *Behavior Genetics, 35,* pp 805-6.

Riemersma, R.F., Van Hutten, R.S., Kalis, A., **Hoekstra, R.A.**, Hoogendijk, W.J.G., Scherder, E.J.A., Swaab, D.F., Van Someren, E.J.W. (2002). Indirect bright light therapy decreases sleep-fragmentation in institutionalized demented elderly; *Neurobiology of Aging Suppl., 23 (1S),* S127.

Van Hutten, R.S., Riemersma, R.F., Scherder, E.J.A., Hoogendijk, W.J.G., **Hoekstra, R.A.**, Berdowski, J., Swaab, D.F., Van Someren, E.J.W. (2000). Frontal task performance in demented elderly is related to the circadian amplitude in the activity rhythm. *Journal of Sleep Research Suppl.,* p. 159.

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