CHAPTER 9

SUMMARY AND GENERAL DISCUSSION
his thesis describes the study of individual differences in autistic traits and withdrawn behaviour and the development of cognitive abilities.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
(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. About half of the genetic variance in autistic traits was shared with variance in WB and SOC scores; the remaining variance was 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...
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 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...
The covariance between verbal and nonverbal abilities was entirely accounted for 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
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 suggest 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 characterized 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).
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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Note: * significant at p<.05 level; ** significant at p<.01 level
References


**SUMMARY AND GENERAL DISCUSSION**