Genetics of Structural Brain Development and Cognition in Childhood and Early Adolescence

Inge van Soelen

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Genetics of Structural Brain Development and Cognition in Childhood and Early Adolescence

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promotoren: prof.dr. D.I. Boomsma
prof.dr. H.E. Hulshoff Pol
copromotor: dr. R.M. Brouwer

Voor mijn ouders

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Introduction

General introduction

The development of the human brain is a lifelong process. From conception to birth the developmental processes that form the basis of the neural network are immense (e.g., Rao and Jacobson, 2005). After birth, brain differentiation and growth continue at a rapid pace in the first years of life. Thereafter the brain undergoes dynamical changes in anatomy and function throughout adulthood into old age (Sowell et al., 2004; Stiles and Jernigan, 2010), but the most pronounced postnatal changes seem to happen around the period of adolescence, when the brain undergoes dramatic remodelling and sculpting. This has been a major focus of research.

The terms "puberty" and "adolescence" are often used simultaneously to refer to the period of transition from childhood into adulthood. In fact, they are distinctive terms for different processes. Adolescence refers to the maturation of social en cognitive behaviours, while puberty represents the period during which individuals become capable of sexual reproduction (Sisk and Zehr, 2005). While some decades ago, the brain of an adolescent was still considered comparable to an adult brain, this vision has been revised intensively due to the development of non-invasive neuroimaging techniques, and in particular due to the use of magnetic resonance imaging (MRI). These techniques allowed for better understanding of neurodevelopmental changes, and brain maturation processes which have initiated a complete new field of research on the neurobiology of the adolescent brain. It has given important insights in the neurobiology of different disorders like Attention-Deficit/Hyperactivity-Disorder (ADHD), autism, childhood psychosis, and (childhood-onset) schizophrenia. Nowadays, many of these disorders are viewed as a result of aberrant developmental trajectories (Karmiloff-Smith, 2010; Insel, 2010; Shaw et al., 2010). In addition, the majority of these disorders have a genetic origin (Sullivan et al., 2003; Muhle et al., 2004; Faraone et al., 2005), and genes that are associated with the disorders have been implicated in developmental processes. For example, many of the risk alleles that are now associated with schizophrenia are involved in processes like neuronal proliferation, migration, or synapse forming (Walsh et al., 2008).

To understand disrupted brain development, we must first understand what normal or healthy brain development is (Luna and Sweeney, 2001), and how healthy brain development is influenced by genotype and environmental exposure. This thesis aims to study how genetic and environmental influences affect individual differences in developmental changes in brain structure and cognition during the transition from childhood to adolescence. This first chapter provides a description of normative brain development and the changes in cognitive performances that occur during childhood and adolescence. The importance of understanding normative brain development is illustrated by the comparison of disrupted development of the brain and cognition, as a consequence of preterm birth and in different psychopathologies that exhibit their first episodes or symptoms around the transition from childhood to adolescence. This chapter concludes with an overview of the literature on twin studies exploring the effects of genes and environment on cognition and brain anatomy in adolescence, to illustrate how genetic studies give more insight into the process of brain development and the underlying mechanisms behind psychiatric disorders.

1.1 Brain development

Although overall brain size already reaches around 80-90% of its adults size at the age of 6 years (Giedd et al., 1999; Paus, 2005), the human brain undergoes considerable changes in structural features and functional organization throughout childhood and adolescence.

Development of brain structure

During adolescence, complex, non-linear, and region specific structural changes occur in the human brain. Total cerebral volume shows an increase, and reaches a plateau around the age of 12 (Lenroot et al., 2007; Tiemeier et al., 2010). The cerebellum shows a later peak in volume compared to the cerebrum, suggesting a prolonged maturation process (Tiemeier et al., 2010). White matter tissue, which is made up of myelinated axons that facilitate the communication between different regions of the brain, shows an almost linear increase in volume with age, and continues to increase in adults until approximately 45 years (Bartzokis et al., 2001). Gray matter tissue, which consists of all the neurons and other supporting cell-types, first increases in volume in childhood and after peaking around puberty, starts to decrease (Paus, 2005; Giedd et al., 2007).

The moment that gray matter volumes start to decrease differs between lobes, revealing a region specific pattern. The frontal and temporal lobes show a gray matter volume peak around age 11. Parietal and occipital lobes show an earlier peak, or already start decreasing in volume at a younger age (Giedd et al., 1999; Lenroot et al., 2007). Decrease in gray matter volume is most often associated by overall thinning of the cortex or changes in cortical gray matter density, which measures the relative gray matter concentration in that specific region (Sowell et al., 2002; Gogtay et al., 2004). Also, differences in timing and the amount of thinning or decrease in density is evident across different areas. On average, gray matter cortical changes appear to occur first in primary cortical areas (e.g., sensory-motor cortex), followed by secondary, and eventually the multimodal cortical areas throughout childhood and adolescence (Sowell et al., 2002; Gogtay et al., 2004).

The biological processes underlying these structural changes around puberty in gray and white matter are only scarcely understood. One of the hypotheses of gray matter loss represents the process of synaptic pruning (Huttenlocher and Dabholkar, 1997). Under influences of genes or environment, unnecessary or unneeded synaptic connections are eliminated. Others argue that the amount of observed gray matter decreases by imaging studies can not be explained by the process of pruning only (Paus, 2005). Decrease in gray matter and the increase in white matter is also thought to be a reflection of progressive age-related axonal myelination (Yakovlev and Lecours, 1967; Benes et al., 1994). In this situation axons that are not yet myelinated are wrongly classified as gray matter at an earlier age. There are different studies that try to disentangle the mechanisms of gray matter decrease or the increase of myelination (e.g., Tamnes et al., 2010), but it remains difficult to really differentiate between the two processes. Regardless of the causes, increase in white matter volume combined with the decrease of gray matter volume, as well as thinning of the cortex is widely accepted to represent brain maturation.

Puberty and the brain

The emergence of puberty is associated with structural and functional brain changes. For example, the start of gray matter decrease is observed around the period of puberty (Giedd et al., 1999). Girls reach the peak in gray matter volume approximately 1 or 2 year earlier than boys (Lenroot et al., 2007), which corresponds with the earlier start of puberty in girls (Marshall and Tanner, 1969; Marshall and Tanner, 1970). However, longitudinal studies on healthy brain development that accurately measure puberty are scarce (Blakemore et al., 2010; Peper et al., 2011).

The start of puberty begins with the moment of the (re)-activation of the hypothalamic-pituitary-gonadal (HPG) axis. After activation in the prenatal period, when the HPG axis is involved in processes for sexual differentiation (Grumbach, 2002), the HPG-axis activity is decreased after birth up to the prepubertal period. When the HPG axis is activated at the start of puberty, the release of Gonadotropin Releasing Hormone (GnRH) is stimulated and induces in turn secretion of Luteinizing (LH) and Follicle Stimulating Hormones (FSH) from the pituitary. LH and FSH activate the maturation of the testes and ovaries and are also associated with the production of the sex steroids testosterone and estrogen, which in turn enhance the maturation of secondary sexual characteristics (Delemarre-van de Waal, 2002; Sisk and Foster, 2004). There are different ways to measure pubertal development (Dorn et al., 2006), for instance measuring hormonal values in morning urine or saliva (Riad-Fahmy et al., 1987; Read et al., 1990; Walker et al., 1990; Demir et al., 1996). Another method is staging puberty by Tanner criteria (Tanner, 1962). This method involves characterisation of the pubertal changes in breast development in girls, genital development in boys and pubic hair growth in both (Tanner, 1962). Although it is assumed that puberty and the increase in sex steroids are linked to brain development (Giedd et al., 2007), only a few studies have looked at the actual association. In these studies some association between brain structure and hormonal levels of LH, testosteron and estrogen were established (e.g., Peper et al., 2008; Peper et al., 2009a; Neufang et al., 2009; Peper et al., 2010). For a more elaborated review and discussion on these findings, see Peper et al. (2011), who concluded that the role of puberty and the associated fluctuations in hormonal levels are a focus for further developmental studies.

Development of cognitive abilities and brain function

General cognitive ability, often assessed with psychometric intelligence quotient (IQ) tests, is remarkably stable across the lifespan (Deary et al., 2000; Bartels et al., 2002; Livingston et al., 2003; Hoekstra et al., 2007; Lyons et al., 2009), and has a high predictive value for several outcome factors in life, such as educational attainment, income, health, and lifespan (Deary et al., 2005; Deary, 2008; Batty et al., 2007; Strenze, 2007). Specific cognitive skills develop considerable during adolescence. For example, the ability for abstract thinking, logical reasoning, or to evaluate hypothetical situations increases (Steinberg, 2005). There are also developmental changes in a variety of information processing abilities, like processing efficiency, or processing speed (e.g., Demetriou et al., 2002). Increases in working memory performance are widely observed from early to mid-adolescence (Gathercole et al., 2004), partly overlapping with increased performance on verbal learning tasks (van den Burg and Kingma, 1999). The development of performance on different cognitive domains seems to be an age-related process, which can be different for specific abilities (Demetriou et al., 2002; Keating, 2004). For example, processing speed performance at age 15 is comparable to that of an adult, while working memory performance does not reach the adult performance level until the age of 19 (e.g., Luna et al., 2004).

The mechanisms linking the structural brain changes and the development of specific cognitive abilities are still not fully understood. Shaw and co-workers showed that cortical thickness trajectories between childhood and adolescence (ages 4 up to 17 years) differed based on the level of intelligence, giving one of the first indications that the developmental trajectory of cortical thickness is associated with cognitive ability (Shaw et al., 2006). The performance on different cognitive tasks indicative for executive control, was associated with the amount of thinning observed in a large cross-sectional sample of children from 8 up to 19 years old (Tamnes et al., 2010). How the maturation processes of structural and functional brain changes in adolescents are linked together, and how this reflects or interacts with the improvement of cognitive abilities remains still unclear.

1.2 Disrupted developmental trajectories in adolescence

Many psychiatric disorders become apparent around or right after puberty (Kessler et al., 2007; Paus et al., 2008). Currently, there is a growing interest in the hypothesis that abnormal behaviour or the development of psychopathology might be influenced or maybe even initiated by processes influencing developmental trajectories of the brain (Insel, 2010; Karmiloff-Smith, 2010; Shaw et al., 2010). There are some findings that indicate the existence of precursors in early life that are associated with the development of psychiatric disorders in later life. For example, in adults with schizophrenia, delayed maturation including delayed developmental milestones in the first year (Sorensen et al., 2010), or decreased cognitive abilities in earlier life (van Oel et al., 2002; Woodberry et al., 2008; Reichenberg et al., 2010) were identified as risk factors for schizophrenia.

1.3 Effects of premature birth

Children born very preterm (VPT < 32 weeks) or with a very low birth weight (VLBW < 1500 g), have a higher risk for the development of cognitive, motor, and behavioural problems later in life (Aylward, 2005; Hack, 2006; Reijneveld et al., 2006; Allin et al., 2008; Larroque et al., 2008). Several MRI studies have revealed morphological brain abnormalities associated with preterm birth (see for review e.g., Hart et al., 2008; Weindling, 2010). Reduction of cerebral volume and enlargement of lateral ventricles have been reported in infants with VLBW, compared to full-term infants. Effects of VPT birth and VLBW, are still present in early adolescence and young adulthood (Skranes et al., 2005). For example, at the age of 15, cerebellar volume was reduced in a group of children born VPT compared to that of age–related peers born at full-term (Allin et al., 2001). The developmental changes in cerebellar volume were different between the two groups, when followed up to the age of 18 (Parker et al., 2008). Cerebellar volume was reduced with time in the VPT individuals when they were scanned again at age 18 years, and cerebellar volume remained stable in term-born control subjects (Parker et al., 2008). Differences in cortical thickness have also been found at adolescence between children born preterm and full term controls (Martinussen et al., 2005; Nagy et al., 2010), which was reflected by a thinner cortex in children born preterm. It has been suggested that preterm birth was related to exaggerated cortical thinning in this period. In addition, differences in structural covariance in structural organization between different cortical regions (Mechelli et al., 2005), was reported in 14 and 15 year old adolescents born preterm (Nosarti et al., 2010).

Based on these studies, one can conclude that children born VPT and or VLBW have a higher risk for brain impairments, not only at birth, but also later in life. It is important to take into account that children born VPT and or with VLBW might represent a very specific high-risk group, within the larger group of premature births, including those that had medical complications at birth. Although infants born after 32 weeks of gestation and within the normal range of birth weight (> 1500 g) are considered to be at low-risk, recent studies indicated increased deficits in developmental trajectories in this group as well (Wang et al., 2004; Morse et al., 2009; Petrini et al., 2009).

The exact mechanisms that can cause preterm birth are not fully understood. The prevalence of children delivered preterm and with lower birth weight is higher in multiple pregnancies (i.e., in majority twin pregnancies) compared to singleton pregnancies. It is known that twins go through a period of catch-up growth after birth, and are comparable in weight and height with age-related singletons around the age of 5 (Estourgie-van Burk et al., 2006). Recently, it was found that gestational age has decreased over the past 20 years for twin pregnancies (Gielen et al., 2010). Decreased birth weight was observed for infants born < 32 weeks, but for infants born > 32 weeks of gestation, birth weight increased (Gielen et al., 2010). Understanding the consequences of preterm birth can contribute

to a better healthcare for children born preterm. It could also contribute to the understanding of how preterm birth is linked to disrupted brain development at a later age, and how this is overlapping with other developmental abnormalities observed in several psychopathologies.

Different sorts of disrupted developmental trajectories are observed in different childhood disorders (Gogtay and Thompson, 2010; Shaw et al., 2010). Future research should focus on the developmental changes of the human brain, and the genetic and environmental, including preterm birth, influences that can act upon these changes. It is not a matter of where you are at a specific age compared to others, it is about how you got there or what you will do in the future that will differentiate a healthy from an non-healthy brain (Karmiloff-Smith, 2010). Thus, the ultimate goal is to further explore what the role of genetic and environmental influences on these trajectories is.

1.4 The role of genes on cognition and brain development

Twin studies are a powerful methodology to quantify to what extent genetic and environmental factors influence individual differences in brain development. By comparing the similarities within monozygotic (MZ) and dizygotic (DZ) twin pairs and siblings on a specific trait, the extent of the observed variance can be attributed to genetic or environmental variance. A larger correlation between members of MZ twin pairs compared to DZ twin pairs and siblings indicates genetic influences. Additive genetic effects (A) represent the effects on the phenotype of multiple alleles at different loci on the genome that act additively. The proportion of the total variance that can be attributed to genetic variance is termed heritability. Common environmental influences (C) include all environmental sources of variance that make twins and siblings who grow up within the same family resemble each other, and thus children from different families unlike one another. These influences may include parental characteristics, home environment, nutrition, socioeconomic status or neighbourhood features. Environmental influences that are unique to an individual and not shared with other family members are referred to as unique environmental influences (E) and cause children from the same family to differ from each other. Unique environmental influences also include measurement error (Falconer and Mackay, 1996).

Heritability of cognition

Twin and adoption studies have explored the genetic and environmental influences on general cognitive abilities at different ages. An intriguing aspect of the aetiology of general cognitive ability is the increase in genetic influences with increasing age, while common environmental influences decrease (Davis et al., 2009; Deary et al., 2009; Haworth et al., 2010). In early childhood, genetic influences explain around 20-30% of the total variance of general cognitive abilities. In middle childhood the relative importance of genetic influences increases up to around 40-50% (Bartels et al., 2002; Bishop et al., 2003; Davis et al., 2008). This increase in genetic influences continues and results in heritability estimates of 70% in young adolescence (Bartels et al., 2002), and 70-80% in adulthood (Posthuma et al., 2001; Bartels et al., 2002; Rijsdijk et al., 2002). Longitudinal twin studies generally report that variation in IQ is explained by the same genetic factor across different ages (Boomsma and van Baal, 1998; Bartels et al., 2002; Davis et al., 2008; Davis et al., 2009; Lyons et al., 2009), and by the same common environmental factors acting during childhood (Boomsma and van Baal, 1998; Bartels et al., 2002; Davis et al., 2009).

When verbal (VIQ) and non-verbal IQ (performance IQ; PIQ) were analyzed separately in a longitudinal twin study of 5 to 18 year olds, some differences in genetic architecture emerged (Hoekstra et al., 2007). For VIQ, 28% of the variance was explained by common environment at the age of 5 years and this decreased to 6% at age 12. However, common environmental influences did not contribute to the variance of PIQ at any age. Phenotypic correlations across the different ages in PIQ was entirely explained by genetic influences, while the phenotypic correlation across the ages in VIQ was influenced by both genetic and common environmental factors (Hoekstra et al., 2007).

A specific example of other cognitive domains that shows profound increase in performance with age is memory. Heritability estimates of memory performance also suggest that these estimates differ as a function of age, but data are scarcer than for general cognitive abilities. Verbal learning is a measure for functioning of the phonological loop, where participants have to recall a list of words immediately after an auditory presentation and after a delay period of 20-30 minutes, showed high heritability in young children (Bishop et al., 1996; 2006; Byrne et al., 2006).

Heritability of brain structure

Variation in global brain structures is explained to a large extent by genetic influences (around 70 - 80%) in adults (Baare et al., 2001; Peper et al., 2007; Kremen et al., 2010), and in children and adolescents (Wallace et al., 2006; Schmitt et al., 2007b; Schmitt et al., 2007a; Peper et al., 2009b; Yoon et al., 2010). Although heritability of global volumes was found to be generally high across the lifespan, heritability by age effects were shown in a large pediatric sample ranging from 5-19 years of age. In this study heritability of gray matter volume decreased and heritability of white matter volume increased with age (Wallace et al., 2006).

Heritability for local cortical thickness throughout the cortex was previously reported in children (Lenroot et al., 2009; Yoon et al., 2010), adults (Brans et al., 2010), and using a region of interest approach in middle-aged men (Panizzon et al., 2009; Kremen et al., 2010). When heritability estimates are compared over the different areas of the cortex, overall differences in heritability values across regions can be observed between studies in early childhood, adolescence and adulthood. For instance, one of the studies had the possibility to look at difference in heritability over time because children were included whithin a large age-range from 5 to 19 years (Lenroot et al., 2009). The results suggested that primary cortical regions (e.g., primary motor and somatosensory cortex, posterior temporal and inferior parietal regions, and occipital regions), showed higher genetic influences at a younger age and heritability decreased with age in these regions. In the same period heritability increased in other regions, which are associated with more complex cognitive functions (e.g., dorsolateral prefrontal cortex, superior parietal cortex, and temporal cortex, and in language-related regions including Broca and Wernicke's areas (Lenroot et al., 2009). This is an indirect indication that the increase of heritability seems to be related with the timing of maturation processes.

Studying heritability as a function of age has generated valuable insights, but with cross-sectional data it is not possible to explore if the same or different genes are responsible for heritability at different ages. It is still unknown if there is a stable genetic factor that influences brain morphology throughout development or whether different genes are expressed across age. In adults evidence was found that there are distinct genetic factors working on brain volumes and on volumetric changes (Brans, 2009). This was also the case when local cortical thickness and the change in cortical thickness with increasing age was explored (Brans et al., 2010). Together, these data suggest that genes involved in brain structure itself may differ from genes that influence changes in structure. It is not known to what extent genes influence brain changes during the important period of adolescence.

There are many biological processes that all can have some effect on how large the brain eventually will grow. How genetic influences acting on the development of brain structure and cognition are associated with each other remains not completely understood. Age related changes in heritability can be linked to the timing of gene expression patterns, and this, in turn, might be related to the age of onset of disorders emerging around this period. Identifying the actual genes that are involved in developmental changes of brain structure and function is still an ongoing journey (e.g., Casey et al., 2010).

1.5 Outline of this thesis

The aim of this thesis is to explore how genetic and environmental influences can affect brain maturation and cognitive functioning around puberty. To accomplish this, a large longitudinal study in a population-based sample of children whose parents registered their twins with the Netherlands Twin Register was started in 2005. At baseline, 9-year-old twins and one older sibling per family were included in the study. Three years later, families came back for follow-up testing. A complete description of the data collection and experimental procedures can be found in **chapter 2**.

In **chapter 3**, the long-term effects of pregnancy duration and birth weight on brain volumes and intelligence at age 9 were explored. The aim of this chapter was to assess the effects of gestational age and birth weight on brain volumes and intelligence in a population-based sample of normal developing children at the age of 9 years.

In **chapter 4** the genetic background of individual differences in dynamic measures of verbal learning abilities in childhood was explored. The Rey's Auditory Verbal Learning Task was used. This task includes a list of words that is presented auditory and repeatedly to the children, who had to remember as many words after each subsequent trial as possible. A dynamic measure was constructed which illustrates individual differences in the learning curve of verbal memory functioning in 9 year-old twins and their older siblings. In **chapter 5** the effect of genes and environment on verbal and nonverbal intelligence scores across two age groups was investigated. In this chapter, longitudinal data on intelligence scores are used from 9 and 12 year old twins and their siblings. It was studied to what extent heritability differs for full scale (FSIQ), verbal (VIQ), and performance IQ (PIQ) in childhood (age 9-11 years), and early adolescence (age 12-14 years). The contribution of genetic and environmental influences on the phenotypic correlation across the two age groups for all IQ scales was explored. This study design allowed an examination of the causes of stability across the two age groups for the different IQ scales, in other words, whether or not the same genetic or environmental factors explain variation in intelligence in childhood and in early adolescence.

Going from cognition to brain structure, genetic and environmental influences on volumetric brain changes between the ages 9 and 12 are explored and described in **chapter 6**. Genetic factors acting on volumetric changes were compared between different brain structures, to explore whether there is one overlapping genetic factor acting on volumetric brain changes. Children rapidly increase in height during this period. Therefore, the increase in height was also included to explore how specific the genetic effects are for volumetric brain changes, compared to the overall increase in height. In **chapter 7**, heritability of brain changes is explored on a more local level. In this study, changes in cortical thickness were calculated and the influences of genetic and environmental influences on the amount of cortical changes were investigated. The aim of this study was to explore whether there is one genetic factor that influences cortical thinning in the child's brain or that instead, multiple independent genetic factors are influencing different brain areas and/or are different across time.

In **chapter 8** the association between cortical thickness at ages 9 and 12 and intelligence is further studied in terms of shared genes and environmental factors. The aim of this study is to explore the correlation between cortical thickness and cognitive ability, assessed by IQ, and to investigate to what extent the associations reflect that the same genes influence both traits. In addition, it was studied whether the associations between cortical thickness and intelligence is specific for verbal and nonverbal IQ.

In **chapter 9**, the results of the studies performed are summarized and future implications of these results are discussed.

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2

Data Collection;

Testing Procedures & Sample Characteristics

The data that form the basis of the studies described in this thesis were collected at two points in time. The first data collection (i.e., baseline) was done in the period of 2005 – 2006, around the time that the twins reached their 9th birthday. The second data collection (i.e., follow-up) took place in the period of 2007-2009, around the 12th birthday of the twins. For details of data collection at baseline the reader is kindly referred to the dissertations of M. van Leeuwen (2008) and of J.S. Peper (2008). In this chapter, a detailed description of the complete testing procedures at follow-up will be given, including some descriptives of the complete sample of twins and siblings. Please note that every component included in the experimental procedure at follow-up as described in this chapter was also included in the test protocol at baseline, if not otherwise indicated.

2.1 Participating families

All families were recruited from the Netherlands Twin Register (Boomsma et al., 2006). At baseline 112 families were included in the study. Families that participated at baseline were all personally invited by letter to participate at follow-up, around the time the twins reached the age of 12 (see Appendices I – III for invitation letter and information brochures that were send to the families). Approximately two weeks after the invitation letter, the research coordinator (I. van Soelen for follow-up) contacted the families by phone. Exclusion criteria were having a pacemaker or metal materials in the head, except for dental braces, chronic use of medication, a major medical history, psychiatric problems as reported by the parents, participating in special education, or physical sensory disabilities. When families agreed to participate, they received approximately 2 or 3 weeks in advance to their visit, packages with additional information at home. These packages contained a confirmation letter for the parents with additional information such as route to the hospital and schedule of the testing day (see Appendices IV and V). The packages for the children contained a confirmation letter as well, and tubes and written instruction for the collection of morning urine and saliva (see Appendices VI - VIII).

In total 89 families (261 children) agreed to participate at the follow-up, resulting in a response rate of approximately 80% of all the families that participated at baseline. Of the 23 families that did not return, the most important reasons for families to refuse were; no time, too much effort (N = 8); some or all family members did not want to participate (N = 11); families that moved to another country (N = 2); and families that moved and new contact details could not be retrieved in time (N = 2).

The study was approved by the Central Committee on Research involving Human subjects of The Netherlands (CCMO), and experiments were performed in accordance with the Declaration of Helsinki. Parents and the children themselves signed informed consents (see Appendices IX and X). Parents were financially compensated for travel expenses, and the children received gift vouchers of 15 euro each at the end of the testing day. In addition, a selection of test scores of each child and a printed image of their MRI scan, when available, was send to their home address afterwards.

2.2 Experimental procedures

The follow-up data collection took place at the University Medical Center in Utrecht (UMCU) and took one day. Please see Table 2.1 for the complete testing schedule and the approximate starting times for the different sections. Families were welcomed at the UMCU approximately at 9 o'clock in the morning. In the first half of the day most of the neuropsychological testing was completed, with two short breaks

Table 2.1 Test protocol and approximate starting time of the different sections.

	Starting time	**Remarks**
Welcome at the UMC Utrecht	9:00 AM	
Collection of questionnaires, saliva, urine, and DNA samples.	9:00 AM	All together
Visit dummy scanner (if necessary)	9:15	All together
Start psychological test protocol		
WISC-III; Intelligence test	9:50	Separately
Break	10:35	All together
N-back task	10:50	Separately
Corsi Block Tapping Task	11:10	Separately
Verbal Fluency	11:20	Separately
Stroop	11:25	Separately
Break	11:30	All together
Rey's Auditory Verbal Learning Task (part I)	11:45	Separately
π-Inspection task	11:50	Separately
Short questionnaire on smoking, drug, skipping school/ classes	11:55	Separately
Rey's Auditory Verbal Learning Task (part II; delayed recall)	12:05	Separately
Lunch break	12:10	All together
Raven Standard Matrices (no time limitation)	12:50	Separately
Start MRI scan protocol (50 min. per person)	14:10	In turns
Physical examination (height, weight, reading test and Tanner assessment)	*In between MRI scans*	Separately
Participants leave the UMC Utrecht	± 16:15-16:30	

All together = children and parents together; Separately = each child separately with a test administration

during which drinks and snacks were available. After lunch, the neuropsychological test battery was completed. For the children who did not have any metal in or around their body that could intervene with the MRI scanner (e.g., metal braces), the afternoon was scheduled for a visit to the MRI scanner. Children were scanned in turns, and while waiting for each other, a physical examination of the children was done in a separate room. The different components of the testing protocol are described in the following sections in more detail.

2.3 Neuropsychological testing

Intelligence

All participants were tested by a series of subtests from the Wechsler Intelligence Scale for children –Third version (WISC-III; Wechsler et al., 2002). The following subtests were included: four verbal subtests (similarities, arithmetic, vocabulary, and digit span), and two nonverbal subtests (picture

completion, and block design). For the calculation of the raw scores on full IQ scale and on the verbal, and performance scale a correction was used to account for the number of excluded subtests. Raw scores were standardized according the age of the child at the time of testing, based on a population sample of same-aged subjects in the Netherlands (Wechsler et al., 2002).

The children also completed the Raven Standard Progressive Matrices (Raven, 1960) which consist of non-verbal multiple choice measures of reasoning and which assesses a measure of general intelligence. The twins' parents completed the Raven Advanced Progressive Matrices (Raven et al., 1994). Data from spouses offer the opportunity to assess the degree of assortative mating for intelligence. It was emphasized that this research was focussed on how much they were alike and not how well they did.

N-back

Children performed a spatial variant of the *n*-back task to assess working memory (Gevins and Cutillo, 1993;Jansma et al., 2000). The task was adapted to make it more appealing for young participants and with increasing levels of difficulty (van Leeuwen et al., 2007). The children had to look at an apple on a computer screen with four holes where a caterpillar would appear. They were instructed that they had to catch the caterpillar by pushing on the corresponding hole. The response buttons were spatially similar to the holes in the apple on the screen. Children were instructed to respond after a warning sound followed by a period that enabled them to press a button before the next caterpillar appeared. Each level of difficulty started with a practise session, only when the participants understood the task it was followed by 3 blocks of each 20 trials. First, one move back was used for practise purposes only, followed by two moves back (N = 2) and finally three moves back (N = 3). The children got feedback on the number of correct responses (whole apple) and incorrect answers (eaten apple) at the screen after each block of 20 trials. Performance was scored as the summed number of correct responses for n-back 2 and n-back 3 separately.

Stroop task

The Stroop Colour Word task (Stroop, 1935) assesses selective attention and consists of 3 components. There are 3 different 'cards' and each card has 10 rows with 10 items printed on it. The children were asked to name the items on each card as quickly as possible. The first set of items consists of the words *'green', 'yellow' , 'red',* and *'blue'* printed in black ink. The second card consists of printed squares in one of the previously named colours. The third card consists of words of names of colours printed in incongruent colours. For this last card children were instructed to name the colours of the words that were printed and not the words itself. Performance on each card was scored as the time needed to read the complete card and the number of mistakes made (i.e., incorrect answer or skipped items). The measure of *Stroop Interference* is calculated by taken the difference in time between the second and third set of items.

Corsi blocking tapping task

The Corsi blocking task measures short-term spatial memory. Nine white blocks distributed unevenly across the computer screen were presented to the children. A number of these blocks turned red for one second each, after which the screen went blank for three seconds. When the

block appeared again on the screen, the children were instructed to select the blocks that previously turned red in the same sequence. The computer registered each response of the child. The task always started with two practice trials. Directly after the practise trial the actual test was started with a series of two blocks. After every 5 trials, the item length increased with one block. The test stopped automatically when the child responded incorrectly for three out of the five trials. Performance on the Corsi task was measured by the total number of correct trials.

Verbal fluency

The children were instructed to name as many words as possible starting with a given letter or included in a given category within a limited amount of time. There were two letter categories, namely '*r*' and '*t*', and two subject categories, namely '*animals*' and '*professions*'. Performance was the total number of correct words given by the child within one minute per category.

Rey's Auditory Verbal Learning Task

The Dutch version of the Rey's Auditory Verbal Learning Test (AVLT) was used (van den Burg and Kingma, 1999), and this task measures both short and long-term verbal memory. It contains 15 unrelated, concrete nouns and was presented to the children by a neutral computerized voice over 5 identical trials. After each presentation the child was asked to recall as many words as possible (part I). After a delay of 20-30 minutes, the participants were asked to recall as many words as possible (part II). Performance was measured after each trial by the number of correct words. Two different versions were used at baseline and follow-up.

π Inspection task

The *π* inspection task measures the speed of processing and was designed after Luciando, et al., (2001). Children had to identify the longer of two lines presented for a short time on the computer screen in the shape of "π". The children were told that the two vertical lines were two worms that disappeared quickly in the ground, and that they had to catch the longest worm to go fishing. Every trial started with a fixation cross in the centre of the computer screen for 1 second accompanied by an alerting beep that sounded for 100 ms, followed by a blank screen. The π figure was then presented (duration was between 2.4 – 2000 ms), and the first response of the child was recorded (i.e., left or right). After five correct answers, a picture of a fish appeared at the button of the computer screen. The children were instructed that it was important to be accurate and catch as many fish as they could, and that it did not matter how long it took them to give an answer. The probability of the longer line appearing at the left and right side was equal. After the stimulus a dynamic mask, consisting of two vertical lines shaped as lightning bolts immediately followed the stimulus and was presented for a period of 300 ms to limit further stimulus processing (for details see Luciando, et al., 2001). For every four correct responses the stimulus duration was decreased for the following trial, and for every incorrect response the duration was increased step by step depending on previous performance. When minimal stimulus duration was reached, the protocol stopped. The protocol was also stopped if the minimum was not reached within 96 trials. On each trial the stimulus duration and the correct or incorrect answer were stored.

Reading ability

The children were asked to read out loud as many words as possible from a card in one minute. The list of words, which consisted of a maximum of 120 words, was adapted from the so-called "Three Minutes Reading Task", which is frequently used in the Netherlands educational system (Cito, 1995).

Handedness

Handedness was determined based on the Edinburgh Handedness Inventory (Oldfield, 1971). In addition a lateralization ratio was calculated at follow-up only, to assess the lateralization by a continuous variable (Leask and Crow, 1997).

2.4 Behavioural data

To all parents of twin pairs registered at the NTR, questionnaires were mailed which included questions about behaviour and health at the ages 0, 2, 3, 5, 7, 10, and 12 years of the twins (Boomsma et al., 2002). The first questionnaire after registration in the twin register, included questions on birth weight, pregnancy duration, and possible pregnancy and birth complications, medication, smoking and alcohol use during pregnancy (van Baal and Boomsma, 1998). At age 2, data on growth, ages (months) when different motor milestones were achieved, and problem behaviour were collected. From this age onward, the questionnaires were mainly focussed on the development of problem behaviour. At age 3, the questionnaires included the CBCL2-3 (Child Behaviour Checklist; Achenbach, 1992; Koot et al., 1997), and at age 5, questionnaires were based on selection of items of Devereux Child Behavior Rating Scale (van Beijsterveldt et al., 2004). At the age of 7, 10, and 12 the CBCL4-18 questionnaires were used (Achenbach, 1991).

At baseline and at follow-up, mothers were asked to fill in similar questionnaires for the siblings, the version corresponded with the age of the child at that moment. At follow-up, the twins and their siblings themselves were asked to fill out the Dutch health and Behaviour Questionnaire (DHBQ) a self-report survey including the Youth Self Report (Verhulst et al., 1997; Achenbach and Rescorla, 2001), to asses problem behaviour, self esteem, life events, religiosity, happiness, life satisfaction, family situation, and family functioning (Bartels et al., 2007). The DBHQ was mailed to the twins and sibs before their visit to the lab, so that they could complete it at home.

2.5 DNA and hormone sample collection and physical examination

In the week before the testing day, the children collected samples of saliva and morning urine (see Appendix VII). Children were instructed to store all collected samples in the refrigerator . All samples were handed in at arrival at the UMCU and were stored in a refrigerator. Hormone levels were determined in saliva (for cortisol and testosterone) and urine (for LH, FSH, and estrogen). The collection of saliva is an attractive alternative to the more conventional procedures because of the ease of frequent collection (Riad-Fahmy et al., 1987). Multiple determinations at a consistent time of the day are required to decrease the influence of fluctuations in hormonal levels, particularly in children (Riad-Fahmy et al., 1987; Read et al., 1990; Walker et al., 1990). Therefore, the children

were instructed to collect all the samples on two school days to restrict the awakening time and time of sampling, because in the Netherlands the starting time of primary school is approximately similar throughout the country. Each participant was asked to write down the exact sample time in a time schedule and to note any exceptional events interfering with normal daily routine.

Both parents and children collected buccal swabs for DNA isolation (Appendix VIII). Subjects were asked to take 2 series of swabs on 2 days, by rubbing 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 prior to buccal cell collection.

Assessment of hormone levels

Hormone levels were assessed by the endocrinological laboratory of clinical chemistry of the VU Medical Center in Amsterdam. Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) levels were measured by an immunometric luminescence assay (Architect, Abbott Laboratories, Diagnostics Division Abbott Park, Illinois, USA). Lower limitation of detection was for FSH 0.11 U/L and for LH 0.1 U/L. Inter-assay for FSH 6% and 5% for 5 and 18 U/L respectively, and for LH 7% and 6% at 4 and 23 U/L. Urinary FSH and LH levels were divided by the creatinine levels to correct for variation in urine excretion levels. Correcting for creatinine levels has been demonstrated to enhance the detection of LH-surges (Kesner et al., 1998). Levels of estrogen were determined in first morning urine samples (competitive immunoassay luminescence assay; Architect, Abbott Laboratories, Diagnostics Division Abbott Park, Illinois, USA). The intra-assay and inter-assay coefficients variation (CVs) were 5% and 10% respectively at levels > 150 pmol/L (lowel limitation of detection) and < 90000 pmol/L (upper limitation of detection). Urinary estrogen levels were also divided by the creatinine levels to correct for variation in urine excretion levels. Testosterone levels in saliva samples were determined by immunometric/competitive immunoassay (lumininescention) assay (ILB, Hamburg). The lower limit or detection was 11 pmol/L and inter-assay variation was 25%, 15% and 10% for < 20, 50, and 75 pmol/L respectively.

Cortisol levels

Children were asked to collect saliva at five time points during the morning using the salivette sampling device (Starstedt, Rommelsdorf, Germany). The first sample was taken in the morning just before getting up from bed, followed by three samples at 15, 30 and 45 minutes after getting up. At noon the last sample was taken. Samples were spinned for five minutes at 3300 rpm and cortisol levels were determined by doing a time-resolved fluorescence immunoassay. The analyses were carried out at the department of Psychology, Dresden University of Technology, Dresden, Germany.

Physical examination

Pubertal stage was determined by a trained project supervisor (J.S. Peper at baseline; I.L.C. van Soelen at follow-up), on the basis of secondary sexual characteristics using the five stages of development devised by Tanner (Marshall and Tanner, 1969; Marshall and Tanner, 1970).

Table 2.2 Scan acquisition details for the scan protocol at follow-up.

MR scan	Acquisition details	Duration (min.)
Scout scan	Sagital T1 weighted; TR = shortest; TE = 20 ms	1 min.
Dual Echo	Dual Echo – Turbo Spin Echo (DE-TSE) clinical scan, transversal T2 weighted; TR/TE shortest / 9, 100; 17 slices of 5 mm; slicegap 1.2 mm; flipangle 90º; FOV: 250 mm / 80%	2 min.
T1-weighted	Three Dimensional - Fast Field Echo (3D-FFE) T1 weighted scan; coronal; 256 × 256 matrix; 160–180 contiguous slices of 1.2 mm; TR = 30 ms; TE = 4.6 ms; flipangle = 30 º ; FOV: 256 mm / 70%;	13 min.
T2-weighted	Dual Echo – Turbo Spin Echo scan using SENSE, transversal T2 weighted; parallel imaging, sensefactor 2; TR/TE1/TE2 6000/15/80 ms; 120 slices of 1.6 mm; slicegap 0.0 mm; flipangle 90 º ; FOV: 250 mm / 80%;	5 min.
DTI	Diffusion Tensor Imaging (DTI) scan using SENSE coil; 15-64 Directions; b-factor 1000; 60 slices of 2.5 mm; slicegap 0.0 mm; 96 × 96 acquisition matrix; flipangle 90 º ; FOV: 240 mm; TE = 60–88 ms; Cardiac gated.	12 min.
MTI	Magnetic Transfer Imaging (MTI) scan; 60 transverse slices of 2.5 mm ; slicegap 0.0 mm; 128 × 96 acquisition matrix; flipangle 8 º ; FOV: 240 mm; TE = 4.5 ms ; TR = 37.5 ms.	2 min.
RS fMRI	Resting State Scan using SENSE coil; parallel imaging, sensefactor 1.8; 3D T2* weighted scan; Time series 800-1200 scans, single scan duration 0.5-0.7 sec; Scan orientation sagittal; 64 × 64 acquisition matrix; 36 slices; FOV 256 mm; 4 mm isotropic voxels; TR/TE 21/31 (shifted echo).	10 min.

Note; All scan parameters were the same at baseline and follow-up. Only the T2-weighted image and the resting state functional MR scans were included at follow-up only.

Characterizations of the pubertal status were assessed by breast development in girls, genital development in boys and pubic hair growth in both (Tanner, 1962). Tanner stage is divided on a 5 item scale. Stage 1 represents no pubertal development and full maturation is represented as stage 5. If children did not feel comfortable with this assessment done by the research supervisor, they were asked to point out their status based on black and white photographs of the different puberty stages, which were accompanied by oral explanation of the researcher. Body weight and height were assessed for each child, and girls were asked about menarche, regularity of their menstrual cycle, and the use of oral contraceptives.

2.6 MRI scan protocol

Scanning and the subsequent image processing took place at the University Medical Center Utrecht, The Netherlands. For both baseline and follow-up the same scan sequences parameters were applied to limit possible differences in scan acquisition between baseline and follow-up (see Peper et al., 2008). For the children who did not have metal braces, or other metal in their body that could intervene with the MRI scanner, the morning started with a practice session in a dummy scanner to get used to the noises of the 'real' scanner (Durston et al., 2009).

Imaging acquisition

A whole head MRI scan was acquired at a 1.5 Tesla Philips Achieva scanner (Philips, Best, the Netherlands). Different sort of scan sequences were included in the scan protocol. All scans at follow-up were the same as at baseline, only at follow-up a resting state fMRI and T2-weighted image were added to the scan protocol. See Table 2.2 for specific scan parameters.

Children were allowed to watch a movie or listen to music during the scans, with the exception of the resting state fMRI scan, where the children were asked to close their eyes and try to avoid thinking of something in particular. The total scan protocol took approximately around 45-50 minutes for each child.

2.7 Sample characteristics

Participants

Mean (s.d.) age of all the participating twins at follow-up was 12.2 (0.3) years, and ranged from 11.7 up to 13.9 years. There were 40 male MZ twins, 41 female MZ twins, 34 male DZ twins, 34 female DZ twins, and 30 opposite sex DZ twins. For all the same-sex twin pairs, zygosity by DNA was already determined at baseline. Mean age of the siblings was 14.8 (1.3) years, ranging from 9.8 up to 18.0 years. There were 45 sisters and 36 brothers. Handedness was determined based on the Edinburgh Handedness Inventory (Oldfield, 1971), and 85% of the twins and 90% of the siblings reported to be right-handed.

Baseline IQ scores of the non-responders (i.e., families who did not return at follow-up), were significantly lower than the baseline IQ scores of children that came back at follow-up (mean (s.d.) IQ of non-responders was 96.4 (13.5); responders was 103.3 (14.8)). The mean IQ scores did not differ between the follow-up sample and the complete baseline sample, so that the effects of intelligence of subjects who did not return for follow-up could be considered to be minimal. Data

Table 2.3 Overview of all main output variables collected at both baseline and follow-up and the mean (s.d.) scores achieved by twins and their siblings separately. All cognitive tests, hormonal values, height and weight are given.

		Baseline			Follow-up		
Task or measure used	Main output phenotype	Mean (s.d.) Twins	Mean (s.d.) Siblings	N total (twins / siblings)	Mean (s.d.) Twins	Mean (s.d.) Siblings	N total (twins / siblings)
Cognition							
Intelligence (WISC-III)	Intelligence Quotient (IQ)	99.9 (13.5)	106.3 (16.3)	224 / 102	100.5 (14.2)	104.7 (17.5)	178 / 81
Raven	Number of correct answers	36.6 (7.9)	45.4 (7.2)	224 / 103	47.0 (5.5)	50.8 (5.8)	179 / 81
Working memory (N-back)	Correct answers (N=2)	28.4 (10.4)	38.4 (13.1)	217 / 99	46.1 (12.4)	52.2 (11.1)	165 / 77
	Correct answers (N=3)	25.5 (7.9)	32.6 (8.9)	213 / 98	35.9 (11.2)	42.7 (12.2)	161 / 75
Inhibition (Stroop)	Interference time (sec.)	71.7 (26.5)	48.6 (17.3)	213 / 101	43.7 (17.8)	34.5 (19.3)	177 / 80
Spatial memory (Corsi)	Number of correct trials	12.0 (3.4)	15.6 (3.8)	221 / 101	16.9 (4.0)	19.0 (3.7)	173 / 79
Verbal fluency	Number Letter	12.2 (3.9)	16.3 (4.8)	224 / 102	17.0 (4.9)	20.5 (6.3)	179 / 81
	Number Category	22.2 (5.0)	28.6 (5.7)	224 / 102	29.6 (5.9)	34.7 (7.2)	178 / 81
Verbal learning (AVLT)	Total correct after 5 trials	39.9 (8.2)	48.0 (7.0)	224 / 101	49.4 (7.6)	54.1 (8.0)	163 / 75
	Total correct after delay	8.4 (2.6)	10.3 (2.5)	224 / 101	10.4 (2.4)	11.0 (2.5)	160 / 74
Processing speed	pi-task (number of trials)	93.2 (6.1)	91.5 (8.6)	224 / 101	90. (8.1)	88.5 (9.2)	148 / 63
Reading ability (1min)	Number of correct words	56.9 (19.6)	84.7 (16.3)	209 / 85	85.2 (18.0)	95.0 (15.8)	167 / 76
Physical examination							
Body	Height (cm)	138.8 (5.3)	155.2 (9.2)	218 / 99	152.5 (7.4)	165.9 (8.8)	174 / 78
	Weight (kg)	31.4 (4.5)	44.1 (9.9)	218 / 99	43.7 (7.9)	57.5 (9.4)	174 / 78
	Body Mass Index	16.2 (1.7)	18.1 (2.6)	218 / 99	18.7 (2.5)	20.8 (2.4)	174 / 78

Puberty stage (Tanner)	Penis development (boys)	1.11 (0.44)	1.70 (0.88)	108 / 44	2.13 (0.85)	3.68 (0.98)	83 / 31
	Pubic hair (boys)	1.09 (0.29)	1.77 (0.99)	107 / 44	2.14 (0.93)	3.74 (1.0)	84 / 31
	Breast development (girls)	1.18 (0.39)	2.83 (0.99)	109 / 54	2.94 (1.1)	4.23 (0.87)	86 / 43
	Pubic hair (girls)	1.16 (0.37)	2.83 (1.3)	108 / 53	2.78 (1.3)	4.36 (0.87)	80 / 44
Hormones							
Morning Urine	LH (U/mmol creatinine)	0.03 (0.04)	0.20 (0.20)	113 / 89	0.19 (0.18)	0.27 (0.21)	175 / 76
	FSH (U/mmol creatinine)	0.36 (0.25)	0.68 (0.49)	223 / 101	0.68 (0.44)	0.78 (0.46)	177 / 77
	Estrogen (pmol / mmol c)	121.3 (101.4)	196.1 (166.0)	221 / 98	285.3 (236.6)	401.2 (295.0)	177 / 77
Saliva	Testosteron (pmol/ L)	28.3 (25.1)	64.9 (62.8)	215 / 99	68.6 (74.7)	157.0 (157.5)	175 / 79
Questionnaires							
Child Behaviour Checklist (CBCL)	Age (years) at moment of survey *	10.1 (0.4)	12.1 (1.2)	158 / 92	12.2 (0.3)	14.7 (1.3)	198 / 81
	Internalizing behaviour	4.5 (5.0)	4.9 (4.8)	157 / 89	4.0 (5.3)	4.1 (5.5)	196 / 80
	Externalizing behaviour	6.7 (6.6)	6.2 (6.8)	157 / 90	5.3 (5.6)	4.4 (5.2)	196 / 81
Youth Self Report (YSR)	Age (years) at moment of survey	-	-	- / -	12.1 (0.3)	14.8 (1.2)	175 / 81
	Internalizing behaviour	-	-	- / -	8.5 (6.7)	8.4 (7.1)	172 / 80
	Externalizing behaviour	-	-	- / -	8.6 (5.7)	8.4 (6.0)	174 / 81

* At baseline only for the siblings the visit to the testing day was accompanied by questionnaires. At baseline the mean scores on internalizing and externalizing behaviour of the twins are based on the (posted) survey closest in age range to the baseline measurements of the twins.

on socioeconomic status (SES) were available for 104 of all the families. SES was on average 3.40 (1.04). Mean SES of the 23 families that did not return was slightly lower (3.04 (0.98)). Mean SES of the families that came back (N = 85) was 3.49 (1.04). SES level did not significantly (p = 0.07) differ between responders and non-responders.

The main phenotypic outcome measures of the cognitive tests are given in Table 2.3, as well as mean hormonal levels, puberty stage, height, weight, and Body Mass Index (BMI) of the sample. At follow-up, 56% of the boys and 68% of the girls showed development of secondary sexual characteristics, and within these groups the girls were more progressed in puberty stage than the boys. Of the 12-year-old female twins participating in the study, 21 reported menarche, of which 3 reported to have a regular menstrual cycle. None of the female twins used oral conceptive. Of their sisters, 38 reported menarche, of which 32 girls reported to have a regular menstrual cycle, 6 used oral contraceptives.

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3

Effects of gestational age and birth weight on brain volumes in healthy 9-year-old children

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Abstract

Objective To assess the effects of gestational age and birth weight on brain volumes in a population-based sample of normal developing children at the age of 9 years. *Study design* A total of 192 children from twin births were included in the analyses. Data on gestational age and birth weight were reported shortly after birth. Total brain, cerebellum, cerebrum, gray and white matter, and lateral ventricle volumes were assessed with structural magnetic resonance imaging. The Wechsler Intelligence Scale for Children-III was administered to assess general cognitive abilities. Structural equation modeling was used to analyze the effects of gestational age and birth weight on brain volumes. *Results* Shorter gestational age was associated with a relatively smaller cerebellar volume ($p = 0.002$). This effect was independent of IQ scores. Lower birth weight was associated with lower IQ score ($p = 0.03$). Birth weight was not associated with brain volumes. *Conclusion* The effect of gestational age on cerebellar volume is not limited to children with very premature birth or very low birth weight, but is also present in children born > 32 weeks of gestation and with birth weight > 1500 g.

Introduction

Several studies have indicated that children born very preterm (VPT; < 32 weeks) or with a very low birth weight (VLBW; < 1500 g) have a higher risk for the development of cognitive, motor, and behavioral problems at a later age (Aylward, 2005; Hack, 2006; Reijneveld et al., 2006; Larroque et al., 2008). Most studies focus on long-term outcome in this high-risk group of infants born VPT. However, infants born between 32 and 37 weeks (also referred as late-preterm or low-risk infants) have an increased risk of deficits in neurological development, development delay, and school-related problems at the age of 5 years (Wang et al., 2004; Morse et al., 2009; Petrini et al., 2009). Earlier studies also indicated that the influence of preterm birth on intelligence is not limited to infants with VLBW. Within the normal range of birth weight (BW), childhood IQ scores showed an increase with higher BW (Boomsma et al., 2001; Matte et al., 2001; Broekman et al., 2009). The results of several magnetic resonance imaging (MRI) studies have revealed morphological brain abnormalities associated with preterm birth (Hart et al., 2008).

Overall, reduction of cerebral tissue and enlargement of the lateral ventricles has been reported in infants with VLBW compared with term infants (≤ 37 weeks) who underwent scanning at approximately the 39th and 41st week of gestation (Inder et al., 2005; Thompson et al., 2007). Furthermore, cerebellar volume reduction has been associated with VPT birth when compared with full-term control subjects at term (Limperopoulos et al., 2005). Other studies only found a decrease in cerebellar volume that was related to the presence of white matter injury in preterm infants compared with control subjects (Shah et al., 2006; Srinivasan et al., 2006).

In individuals with a history of preterm birth, brain tissue differences are still present in early adolescence and adulthood (Nosarti et al., 2002; Allin et al., 2004; Fearon et al., 2004; Skranes et al., 2005; Allin et al., 2007; Nosarti et al., 2008). At 15 years of age, reduction of cerebellar volume was observed when comparing VPT born adolescents with full-term control subjects (Allin et al., 2001). Cerebellar volume was reduced with time in the VPT individuals when they were scanned again at age 18 years, and cerebellar volume remained stable in term-born control subjects (Parker et al., 2008). Thus, these studies show an association between preterm birth and disturbed cerebellar development, not only shortly after birth but also in later life.

Most of these studies focussed on infants with VLBW without taking gestational age (GA) into account or vice versa. As a result, these studies also include infants who are small for GA. These infants have an increased risk for impaired developmental outcome at later age (Walker and Marlow, 2008). Furthermore, other medical complications at birth and brain injuries are more often present in infants who are VPT and small for GA. These infants could present a specific high risk group within the larger group of all premature births. Although infants born after 32 weeks of gestation and within the normal range of birth weight (> 1500 g) are considered to be a low-risk group, recent research observed increased deficits in developmental trajectories (Wang et al., 2004; Morse et al., 2009; Petrini et al., 2009). We explored the association among brain volumes at 9 years of age and gestational age and birth weight in a sample of 192 preterm and term children. All children were born with a birth weight > 1500 g and without major medical complications.

Methods

Subjects

A total of 224 children from monozygotic (MZ) and dizygotic (DZ) twin pairs were recruited from the Netherlands Twin Register (NTR; Boomsma et al., 2006) at the age of 9 years. The twin pairs all participated in a larger ongoing study to explore the genetic and environmental influences

on individual differences in brain maturation around puberty (van Leeuwen et al., 2008; Peper et al., 2009). Data on GA and BW were obtained with a survey sent to the mothers shortly after birth. Exclusion criteria for participating in the MRI study consisted of having a pacemaker, any metal materials in the head (including dental braces), chronic use of medication, a known major medical problem (e.g., known neurologic problems, physical or sensory disabilities) or psychiatric history (parents or children), and participation in special education.

A total of 207 individuals completed the MRI protocol. One monozygotic twin pair was excluded from analysis because of being VPT on the basis of short pregnancy duration (< 32 weeks). Children with VLBW were excluded (< 1500 g; N = 3). The Wechsler Intelligence Scale for Children-III was administered, and children with an IQ < 70 were excluded (N = 3).

A total of 192 children were included in the analyses (98 female and 94 male). The mean GA of the children was 36.8 weeks (s.d. = 1.6 weeks), with a range of 32.5 to 40 weeks. Seventy-eight children were born preterm, and 114 children were born at 37 weeks gestation or later. The mean birth weight was 2614.6 g (s.d. = 438.6 g), with a range of 1525 to 3820 g. After birth, 91 children remained in an incubator (for an average of 6 days). The mean age of the children at the time of the scan was 9.2 years (s.d. = 0.1 years), with a range of 9.0 to 9.7 years. The mean (SD) of full-scale IQ score was 101.1 (s.d. = 12.9), with a range of 71 to 143. Parents gave written informed consent to participate in the study. The study was approved by the Central Committee on Research involving Human Subjects of the Netherlands, and experiments were performed in accordance with the Declaration of Helsinki.

Scan acquisition

Structural MRI of the whole brain was performed on a 1.5-T Achieva scanner (Philips, Eindhoven, the Netherlands). All children had a practice session in a dummy scanner in advance. During this session, children could familiarize themselves with the scan procedure, small space, and the sounds of the MRI machine. Children were able to watch a movie or listen to music during the scan protocol, which took approximately 35 minutes per child. Image-sequences of the whole head were acquired, including a short scout scan for immediate verification of optimal head positioning, a clinical scan that was used for neurodiagnostic evaluation, and a 3-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head (256 × 256 matrix, TE = 4.6 ms, TR = 30 ms, flip angle = 30º, 160-180 contiguous slices; 1 × 1 × 1.2mm3 voxels, field-of-view = 256mm/70%), which was conducted for volumetric analysis. Additionally, a diffusion tensor image (DTI-B0: transverse; 15-64 directions; SENSE factor 2.5; flip angle 90º; 60 slices of 2.5 mm; no gap; 128 × 96 acquisition matrix; field-of-view = 240 mm; TE = 78 ms) and a magnetization transfer image scan (transverse; 60 transverse slices of 2.5 mm; no gap; 128 × 96 acquisition matrix; field-of-view = 240 mm; flip angle 8º; TE = 4.5 ms; TR = 37.5 ms) were used for segmentation of the intracranial volume (Peper et al., 2008).

Volumetric Measurements

Scans were put in Talairach frame (no scaling) for alignment of brain images and were corrected for inhomogeneities in the magnetic field (Sled et al., 1998). Quantitative assessments of the intracranial (IC) volume and volumes of total brain, cerebellum, cerebrum, gray and white matter of the cerebrum, and lateral ventricle volumes were performed on the basis of histogram analyses and a series of mathematical morphology operations to connect all voxels of interest, as validated

(Schnack et al., 2001a; Schnack et al., 2001b) and reported (Peper et al., 2009). Because of motion artifacts, it was not possible to separate gray and white matter tissue in 11 children. For 10 of these 11 children, it was also not possible to calculate lateral ventricle volumes. In addition, in 2 children only the segmentation of lateral ventricles was missing. As a result, the analyses on gray and white matter volumes included data from 181 children, and the analyses on lateral ventricles included data from 180 children.

Statistical analyses

The effects of GA and BW on brain volumes and IQ were tested with regression analyses. To control for familial dependency, data were analyzed with a structural equation modelling approach. The covariance structure in twin pairs was simultaneously modeled with the fixed effects of BW and GA on mean brain volumes and IQ (Neale and Cardon, 1992). For all analyses, the software package Mx (Myricom; www.myri.com/scs/ download-mx10g.html) was used (Neale et al., 2006).

GA was tested in 2 ways. First, GA was modelled as a continuous variable (in weeks). Second, with dichotomizing GA with a threshold at 37 weeks, group differences on brain volumes and cognitive abilities were explored in children born preterm (i.e., 32.0-36.5 weeks) or at term (i.e., 37-40 weeks). In all tests for brain volumes, IC volume, age at time of scan, and sex were included as covariates. The full regression model to describe the observed variance in brain volumes is given by this equation:

$$\text{Brain volume} = \text{Intercept} + (\beta_{ga}*\text{GA}) + (\beta_{bw}*\text{BW}) + (\beta_{age}*\text{age}) + (\beta_{sex}*\text{sex}) + (\beta_{ic}*\text{IC})$$

All parameters were estimated with maximum likelihood. The effects of GA and BW were tested by constraining the regression coefficient at 0 (i.e., the effect of GA was tested while correcting for effects of BW and vice versa). Comparison of the models was done with likelihood ratio χ tests. These tests compare the differences between –2 * log likelihood (which is χ^2 distributed) of the full model with that of the restricted nested model against the corresponding degrees of freedom.

Results

Means (s.d.) of GA, BW, absolute brain volumes, and intelligence scores are presented in Table 3.1. Brain measures and IQ were all normally distributed, except for lateral ventricle volume. After a logarithmic transformation, the lateral ventricle volumes were normally distributed.

GA and age at the time the MRI was performed were not correlated with each other (i.e., children who were scanned at a younger age were not the children with shorter pregnancy duration; $r = 0.01$, $p = 0.94$). Absolute brain volumes were significantly larger for boys than for girls for all measures. Children born preterm did not differ in IQ compared with children born ≥ 37 weeks ($p = 0.08$).

Results of structural equation model fitting are presented in Table 3.2. First, effect of GA was tested on mean brain volumes and IQ scores. GA had a significant effect on cerebellar volume ($p = 0.002$), indicating that children who were born at a later GA had a larger cerebellar volume at 9 years of age ($\beta = 1.98$ mL/week). The Figure depicts the association between GA and relative cerebellar volume (i.e., corrected for BW, IC volume, age at time of scan, and sex). By dichotomizing GA, cerebellum volume was smaller for children born preterm (estimated value for deviation between groups was 5.7 mL; $p = 0.006$), while correcting for differences in BW, sex, age, and IC

Table 3.1 Mean (s.d.) gestational age (weeks), birth weight (g), age at moment of scan (years), and absolute brain measurements (ml) of the children included in the analyses.

	Mean *(s.d.)*	*(min. – max.)*	**N**
Pregnancy duration *(weeks)*	36.8 (1.6)	*32.5 - 40.0*	195
Birth weight *(grams)*	2613.5 (438.1)	*1525 – 3820*	195
Age at moment of scan *(years)*	9.2 (0.1)	*9.0 – 9.7*	195
Full scale IQ	101.1 (12.9)	*71 - 143*	192
Intracranial volume *(ml)*	1466.2 (126.3)	*1213.1 – 1813.2*	195
Total brain volume *(ml)*	1356.3 (116.0)	*1120.5 – 1641.7*	195
Cerebrum*(ml)*	1192.7 (106.1)	*966.3 – 1458.3*	195
Cerebellum *(ml)*	153.2 (13.9)	*126.3 – 191.9*	195
Cerebrum Gray matter *(ml)*	742.5 (66.9)	*592.8 – 905.4*	182
Cerebrum White matter *(ml)*	451.2 (48.3)	*340.5 – 579.7*	182
Lateral ventricle *(ml)*	9.2 (6.6)	*2.1 – 55.0*	195

volume. No significanct effects of GA were found for the other brain volumes. To explore whether this effect could be explained with differences in general cognitive abilities, intelligence scores were included as a covariate. GA still had a significant effect on cerebellar volume ($p = 0.002$). Thus, the association between GA and cerebellar volume could not be explained by differences in intelligence scores. GA had no significant effect on the other brain measures (i.e., total brain, total cerebral and cerebral gray and white matter, and lateral ventricle volumes).

Second, the effect of BW on brain volumes was tested, while correcting for GA, IC volume, age at scan, and sex. No significant effects were observed for BW on any of the brain volumes. Children born with lower BW had lower scores on the IQ test ($\beta = 0.49$ points/per 100 g; $p = 0.03$). When IC volume was included as a covariate, the effect of BW on IQ score no longer reached significance ($p = 0.30$). To gain a more comprehensive view of this relationship, BW was tested for its effect on IC volume. Higher BW was associated with larger IC volume ($p < 0.01$), corrected for GA, sex, and age at scanning.

Discussion

We explored volumetric brain measurements at 9 years of age in normally developing children with a GA between 32 and 40 weeks and within the normal range of birth weight, from 1525 to 3820 g. Children born preterm had a smaller cerebellar volume compared with children born ≥ 37 weeks. We found that shorter GA was associated with a relatively smaller cerebellar volume at 9 years. These analyses were corrected for BW, IC volume, age at time of scan, and sex. Thus, the reported effects were to a large extent independent of overall head size differences. GA had no significant effect on the other brain volumes or on IQ. We found that lower BW was associated with lower IQ score at age 9 years. When IC volume was included as a covariate, the effect of BW on IQ score did not reach significance anymore. BW had no effect on any of the brain volumes.

Because of the number of tests that we performed, false positive results were possible. Therefore, an adapted significance threshold was calculated to control for the possible presence of multiple testing effects (http://gump.qimr.edu.au/general/daleN/matSpD/). On the basis of the correlation matrix of the variables tested (i.e., brain volumes, IQ), the experiment-wide significance threshold required to keep the type I error rate at 5% should be $p = 0.006$. As a result, the effect of GA on cerebellar volume ($p = 0.002$) is still significant after a stringent correction for multiple comparisons. The effect of BW on IQ ($p = 0.03$) must be interpreted with caution.

The results of the effect of GA on cerebellar volume are in agreement with earlier studies. Reduced cerebellar volume was previously found in preterm and VPT infants (range, 23-37 weeks gestation; mean BW, 1341 g) scanned at term compared with full-term infants (Limperopoulos et al., 2005). In contrast, other studies only found a decrease in cerebellar volume that was related to the presence of white matter injury in preterm infants compared with control subjects (Shah et al., 2006; Srinivasan et al., 2006).

In adolescence, reduction of cerebellar volume was also observed at approximately the age of 15 years, when young adults born VPT (< 32 weeks of gestation) were compared with full-term control subjects (Allin et al., 2001). In VPT children, the cerebellar volume decreased in the period from age 15 to 18 years, while cerebellar volume remained stable in term-born control subjects (Parker et al., 2008). Our results illustrate that the relationship between preterm birth and disturbed cerebellar development is not limited to VPT children, but is also present in preterm children with BW > 1500 g.

Furthermore, our findings support the notion that during the last trimester of pregnancy the cerebellum undergoes a very active state of development (Limperopoulos et al., 2005). It is known that the cerebellum reaches maturity late, comparable with the prefrontal cortex (Diamond, 2000). Recently, the role of the cerebellum has been acknowledged in cognitive and emotional functioning (Limperopoulos et al., 2007; Baillieux et al., 2008; Schutter and van Honk, 2009). Moreover, the cerebellum has been implicated in the pathology of several neuropsychiatric disorders (Hoppenbrouwers et al., 2008).

A lower BW was associated with lower IQ, while correcting for GA, sex, and age. This result is in line with earlier studies reporting that childhood IQ measures are influenced by BW (Boomsma et al., 2001; Matte et al., 2001; Broekman et al., 2009). The effect of BW on IQ disappeared when differences in IC volumes were taken into account. Additional analyses showed that BW had an effect on absolute IC volume, suggesting that the association found between BW and childhood IQ is mediated by individual differences in head size.

Table 3.2 Results of the model fit analyses on the mean volumetric measures at age 9.

		Estimated β	**95% CI**	
		(unstandardized)	*(lower/ upper)*	**p-value**
Cerebellum	Gestational age	**1.77**	*.54 / 3.00*	**.005**
	Birth weight	-.002	*-.006 / .001*	*n.s.*
Cerebrum	Gestational age	-1.45	*-4.08 / 1.15*	*n.s.*
	Birth weight	**.008**	*.0003 / .016*	**.041**

The estimated β value of gestational age and birth weight on cerebellar and cerebral volumes are given with the 95% confidence interval (CI), while correcting for sex, intracranial volume, age at scanning, and birth weight or gestational age respectively; n.s. = not significant.

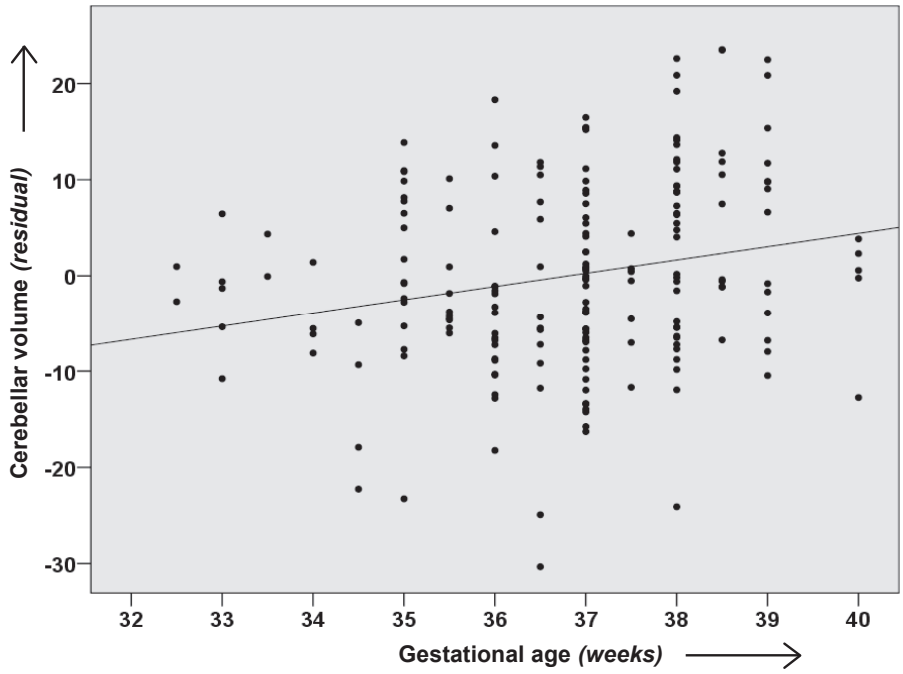

Figure 3.1 Association between gestational age (weeks) and cerebellum volume residual (i.e., cerebellum volume corrected for age at moment of scan, sex, intracranial volume and birth weight) at age of 9. Children who were born at a later gestational age had a larger cerebellar volume at 9 years of age (β = 1.77 ml / week: p = 0.005).

There was no effect of BW on any of the brain volumes at age 9 years. These findings are partly consistent with other MRI studies. Some of these studies report smaller brain volumes in VLBW infants compared with normal BW infants (Nosarti et al., 2002; Inder et al., 2005), but other studies found no effects of BW on total cerebral brain volume (Allin et al., 2001; Allin et al., 2004; Fearon et al., 2004). Although most of these studies did not find effects on total cerebral volume, they do report differences in local gray matter and white matter differences. An important difference compared with this study is that other studies have included VPT infants of < 32 weeks of gestation. Disruption of development or severe injuries within the cerebellum are most prominent in infants who were born at < 32 weeks of gestation (Messerschmidt et al., 2008b) and have been associated with poor neurological outcome at a later age (Messerschmidt et al., 2008a).

Infants born after 32 weeks of gestation or with BW > 1500 g are, in general, considered to be a late-preterm or low-risk group. However, recent research noted that this group of infants has an increased risk of deficits in neurologic development and a higher risk for developmental delay and schoolrelated problems at later age (Wang et al., 2004; Morse et al., 2009; Petrini et al., 2009). This present sample included a wide GA range (32 - 40 weeks) and BW > 1500 g. These birth parameters are within the normal range for average twin pregnancies (the Netherlands Perinatal Registry, 2009; Gielen et al., 2010). More important, although the mean BW of twins is lower compared with that of singletons, there were no children included in this sample who had a VLBW. This study can help to get a better understanding of the developmental trajectory in this group of children.

Data on possible birth complication, pregnancy duration, and BW of the twins were based on questionnaires filled in by the mothers shortly after birth, a method found to be highly accurate (Tomeo et al., 1999; O'Sullivan et al., 2000; Walton et al., 2000). The mothers also reported the number of days twins spent in an incubator after birth. The average period of being in an incubator was 6 days, and this is not uncommon for multiple birth and infants who are born preterm.

A limitation of this study is whether or not these findings in twins can be generalized to the general population, consisting of mostly singletons. Although for most multiple pregnancies, children are delivered preterm and with lower BW compared with singletons, the mechanisms that initiate preterm labor are still not fully understood. Associations are found with maternal factors (e.g., age, weight, socioeconomic status), fetal factors (e.g., sex), and environmental factors (e.g., substance abuse, infection, traumatic life events) (Murphy, 2007; Goldenberg et al., 2008). Genetic susceptibility and interactions between factors also may play a role (Kistka et al., 2008). Twins undergo catch-up growth during childhood, and by the age of 5 years, growth differences between twins and singletons have disappeared (Estourgie-van Burk et al., 2006). In adulthood, cognitive performances and brain volumes are comparable between singletons and twins (Posthuma et al., 2000; Hulshoff Pol et al., 2002). Thus, it might be argued that these results in twins can be generalized to the singleton population.

Another limitation of this study might be the exclusion criteria. Because deficits in cognitive functioning are associated with deficits in brain structure and volumes, it is possible that some of the subjects with the most prominent sequelae of cerebellar volumetric loss are not included in this study. However, this study still exhibits a wide range of individual differences in general cognitive abilities and is a good representation of healthy developing children. It is likely that the observed effect might be even more pronounced if children with known developmental, cognitive, or brain developmental deficits would be included in the analyses.

All children were 9 years old at time of the scan, and age was not associated with GA. The association between GA and cerebellar volumes was not moderated by age at the time of scan, but appears to be very specific for the duration of pregnancy. Recently, GA was found to be decreasing in a linear fashion in twin birth in the last 2 decades, at an average of 0.25 days per year (Gielen et al., 2010). Furthermore, BW appeared to decrease for infants born before 32 weeks of gestation and increase after 32 weeks of gestation (Gielen et al., 2010). Therefore, it is important not to ignore this low-risk group, and future research should focus on their long-term developmental trajectories.

This study showed that younger GA was associated with a relatively smaller cerebellar volume at the age of 9 years while correcting for effects of sex, age at time of scan, BW, and IC volume. Our results extend the relationship between preterm birth and disturbed cerebellar development at birth and later life in VPT children to the group of preterm children. Children with lower BW scored lower on intelligence tests, but this effect disappeared when IC volume differences were taken into account. This study contributes to the understanding of the long-term effects of preterm birth and shows that effects of GA and BW on brain volumes and IQ is not limited to the group of VPT infants or infants born with VLBW. Further research should focus on the long-term outcomes of the developmental trajectories of late-preterm children.

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4

Individual differences in dynamic measures of verbal learning abilities in young twin pairs and their older siblings

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Abstract

We explored the genetic background of individual differences in dynamic measures of verbal learning ability in children, using a Dutch version of the Auditory Verbal Learning Test (AVLT). Nine-year-old twin pairs (N = 112 pairs) were recruited from the Netherlands Twin Register. When possible, an older sibling between 10 and 14 years old participated as well (N = 99). To assess verbal learning, non-linear curves were fitted for each child individually. Two parameters were estimated: Learning Speed (LS) and Forgetting Speed (FS). Larger twin correlations in monozygotic (MZ) than in dizygotic (DZ) and sibling pairs for LS and FS indicated the importance of genetic factors in explaining variation in these traits. The heritability estimate (percentage of variance explained by genetic factors) for LS was 43% for both twins and siblings. For FS heritability was estimated at 20% in twins and was slightly higher (30%) in their older siblings.

Introduction

The ability to store a limited amount of verbal information in highly accessible form over a short period of time, like remembering a shopping list, is an important cognitive ability for both children and adults in everyday life. A specialized component closely linked to these verbal learning abilities, is the phonological loop (Baddeley et al., 1998). The phonological loop can be assessed by the use of verbal repetition paradigms, often called verbal learning paradigms, which may consist of both familiar or non-word series of words (Gathercole et al., 1994). Immediate recall of words is mediated by the semantic similarity between words, word frequency, the order of presentation and is related to long term memory (Cowan, 1988; Burgess and Hitch, 2006; Repovs and Baddeley, 2006; Richardson, 2007).

There are few studies that focus on the etiology of individual differences in verbal learning ability. Learning rates and achievement measures of the 'same' process do not necessarily overlap (Byrne et al 2008). This paper focuses on a possible contribution of genetic factors to variation in the dynamics of verbal learning during childhood. The contribution of genetic factors to trait variation can be estimated by comparing the trait similarity between identical (monozygotic, MZ) and non-identical (dizygotic, DZ) twin and sibling pairs. The proportion of the variance in a trait that can be attributed to genetic factors is termed heritability. For general cognitive ability it is well-documented that heritability increases from around 25% in 5- to 6-year olds to 80% in adults (McClearn et al., 1997; Finkel et al., 1998; Bartels et al., 2002). Heritability estimates for measures of memory performance also suggest that they differ as a function of age, but data are scarcer than for general cognitive abilities.

Verbal learning is often assessed with the California Verbal Learning Test (CVLT) or the Rey's Auditory Verbal Learning Test (AVLT). The CVLT and AVLT include a list of words that is presented auditorily and repeatedly. Participants have to recall the words immediately after each presentation of the list and after a delay period of 20-30 minutes. A study in elderly male twin pairs (mean age 71.8 years), showed a heritability of verbal memory of 56 % (Swan et al., 1999). Other word repetition studies with a list of non-words, showed high heritability in young children (Bishop et al., 1996, 2006; Byrne et al., 2006).

There are a limited number of studies that included a measure of the dynamics during the initial trials of a verbal learning task (e.g., adding and retaining words of a given list into the phonological loop). The most widely used measures of verbal learning are the total number of correctly recalled words or the difference between the correctly recalled words on e.g. trial 5 and trial 1. This is often called the learning slope or learning rate (Vakil and Blachstein, 1993; Paolo et al., 1997; Vakil et al., 2004; Woods et al., 2006). By taking the difference in recalled words between trials, the learning curve is assumed to be linear while a non-linear learning curve might be more appropriate (van den Burg and Kingma, 1999; van der Elst et al., 2005; Poreh, 2005). We apply a method to measure the dynamics during the process of keeping words into phonological loop to data collected in a sample of young twins and their siblings. By using non-linear regression analysis and fitting a learning curve for each individual, two parameters are estimated. The first parameter, Learning Speed (LS), represents the proportion of verbal material not yet recalled in a previous trial that is recalled in a following trial. The second parameter, Forgetting Speed (FS), represents the proportion of material that was successfully remembered previously, that can no longer be recalled in a following trial. By estimating these two indices a more comprehensive picture of the dynamics of verbal learning is obtained.

Methods

Participants

Subjects were recruited from the Netherlands Twin Register (Boomsma et al., 2006) and participated in a larger ongoing study, described by van Leeuwen et al. (2008) and Peper et al. (2008) in more detail. A total of 112 healthy twin pairs (23 MZ male, 25 MZ female, 23 DZ male, 21 DZ female, and 20 DZ opposite sex pairs) and their older siblings (N = 99; 56 female) completed the verbal learning task. Mean age of the twins was 9.1 years, ranging from 8.9 to 9.5 years. Mean age of the siblings was 11.9 years, ranging from 9.9 to 14.9 years. Exclusion criteria consisted of chronic use of medication, any known major medical or psychiatric history, participation in special education or an IQ < 70. Socio-economic status (SES) was slightly above the average of the Netherlands (Statistics Netherlands (CBS), 2004), but still showed substantial variation (low SES, N = 17; middle SES, N = 54; high SES, N = 37; unknown SES, N = 4). Written informed consents were obtained from all subjects and their parents and the study was approved by the Dutch Central Committee on Research involving Human Subjects (CCMO).

Experimental procedure

The Dutch version of the Rey's Auditory Verbal Learning Test (AVLT) was used (van den Burg and Kingma, 1999). It contains 15 unrelated, concrete nouns and was presented to the children by a neutral computerized voice over 5 identical trials. After each presentation the child was asked to recall as many words as possible.

Learning and Forgetting Speed

For each child a non-linear learning curve was fitted using the number of correctly recalled words over the first 5 trials. This learning curve was identified by two parameters, namely Learning Speed (LS) and Forgetting Speed (FS). LS represent the proportion of verbal material not yet recalled in a previous trial that is recalled in a following trial. When LS increase in value, a steeper increase in correctly recalled words is seen over the first 5 trials. FS represents the proportion of material that was successfully remembered previously, that can no longer be recalled in a following trial.

An individual learning curve of the performance from trial 1 to trial 5 was described as: $Y(t) = A * (1 - [1 - b]^{t})$, where Y is the total amount of words recalled, A and b are learning parameters and t is the trial number (Mulder et al., 1996). The formula is derived from the difference equation: $\Delta Y = a - b * Y$, which describes a dynamic growth process. Because the specific task at hand has a fixed amount to be learned, this equation can be reparametrized as: $\Delta Y = a * (15 - Y) - b * Y$, where a and b can be interpreted as LS and FS, respectively (in solving this equation it is assumed, that on trial 0 nothing has been learned, that is $Y_{(t = 0)} = 0$). Both LS and FS were estimated by the Newton-Raphson method for non-linear regression (Dorn and McCracken, 1972).

Genetic modeling

Structural equation modeling (SEM) was used to decompose variance in LS and FS into genetic and environmental variances (e.g., Boomsma and Molenaar, 1986; Neale et al., 2006). These estimations are based on the assumption that monozygotic (MZ) twin pairs are genetically

identical and share (nearly) 100% of their genetic material, while dizygotic (DZ) twin pairs and full siblings share on average 50% of their segregating genes (Boomsma et al., 2002). By adding an additional sibling in the analyses statistical power will increase (Posthuma and Boomsma, 2000). Additive genetic effects (A) represent the effects on the phenotype of multiple alleles at different loci on the genome that act additively. It is possible that effects of alleles do not simple add up and depend on the presence of other alleles, resulting in non-additive or dominance genetic effects (D). This results in a relatively large difference between MZ and the DZ correlations. Common environmental influences (C) include all environmental sources of variance that make twins and siblings who grow up within the same family resemble each other. Environmental influences that are unique to an individual and not shared with other family members are referred to as unique environmental influences (E). These also include measurement error (Falconer and Mackay, 1996). The influences of D and C can not be disentangled when data are collected in twins and siblings raised together. Because we observed MZ correlations more than twice as high as DZ and sibling correlations for both the LS and FS phenotype, an ADE model was fitted to both measures. The ADE model is illustrated in figure 1, where the circles represent latent factors (A, D and E) and boxes the measured trait (LS or FS) of the first twin, second twin and sibling.

If A, D and E are standardized to have unit variance, the total variance of a trait due to A, D and E is given by the square of the factor loadings *a*, *d*, and *e* respectively:

$$\text{Var (P)} = a^2 + d^2 + e^2 \quad (1)$$

The correlation between A_1 (the latent genetic factor for twin 1) and A_2 (twin 2) is fixed at 1.0 for MZ twin pairs. The correlation between D_1 and D_2 is also fixed at 1.0 for MZ twin pairs. For DZ twin pairs and twin-sibling pairs, the correlations between A_1, A_2 and D_1, D_2 are 0.5 and 0.25, respectively (Falconer and Mackay, 1996). Unique environmental influences (E) are by definition uncorrelated in all twin and sibling pairs. The covariance between twins and siblings is given by (see also Figure 4.1):

$$\text{Covariance MZ twins} = a^2 + d^2 \quad (2)$$
$$\text{Covariance DZ twins and siblings} = 0.5 * a^2 + 0.25 * d^2 \quad (3)$$

The software package Mx (Neale et al., 2006) was used to carry out the genetic SEM analyses by fitting equations 1, 2, and 3 to the observed data. In addition, the mean structure of the data was analyzed. In a so-called saturated model (Neale and Cardon, 1992) we compared the means and variances of twins and siblings and of MZ and DZ twins. Twin-twin and twin-sibling correlations and their 95% confidence intervals were estimated for MZ and DZ families. Within this model, regression effects of age and sex on the means were tested for significance. Sex effects on means were explored in two steps. First, sex effects were modeled separately for twins and siblings. Secondly, an overall sex effect was tested for significance.

Next, parameters *a*, *d* and *e* were estimated. All parameters were estimated by maximum likelihood. Models were compared by likelihood ratio tests. By comparing the goodness of fit of nested submodels, where one or more parameters are set at zero or in which parameters are specified to equal other parameters, it is determined which model is the most parsimonious while still describing the data well. The difference in the number of parameters estimated in two nested models gives the degrees of freedom (*df*). When testing whether a parameter is equal to zero, the likelihood-ratio test statistic does not follow a chi-square distribution. The solution is to half the p-values (Dominicus et al., 2006). Therefore, when testing for the significance of *a* and *d*, we report the adjusted p-values.

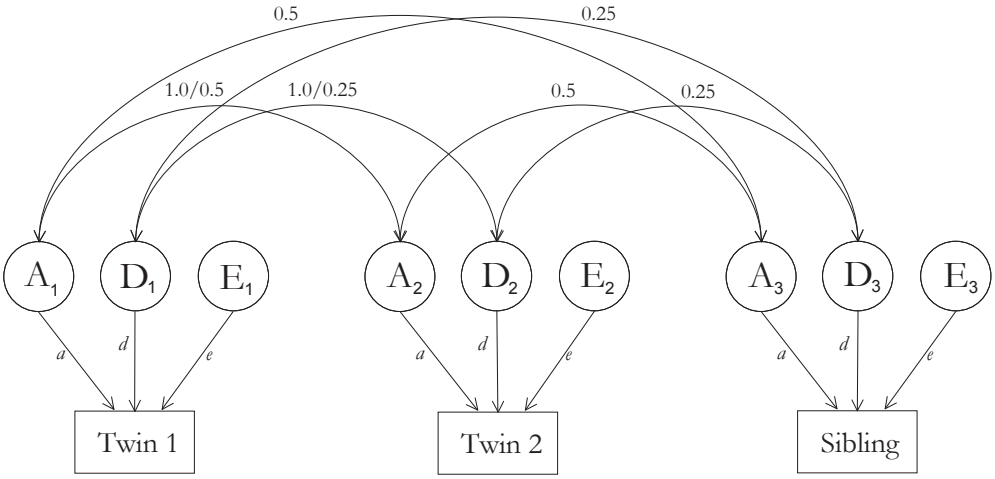

Figure 4.1 Genetic ADE model for the LS and FS including the 9-years old twin pairs and their older siblings. Total variance in twins and siblings is explained by A (additive genetic), D (dominance genetic), or E (unique environment) effects. The covariance of the A component between twins (i.e., A_1 and A_2) or twin-sibling pairs is fixed at 1.0 and 0.5 for MZ and DZ, respectively. For twin-sibling pairs (i.e., A_1 and A_3 or A_2 and A_3) the covariance of the A component is fixed at 0.5. Covariance of D component is fixed at 1.0 and 0.25 for MZ, DZ and twin-sibling pairs respectively. The influences of E is non-shared by family members.

Results

Phenotypic analyses

Figure 4.2 depicts the mean (s.d.) number of correctly recalled words on each of the first 5 trials. The average number of correctly recalled words increased each following trial for both twins and siblings. LS was normally distributed and FS was positively skewed. After a logarithmic (base 10) transformation the distribution of FS was normal.

Table 4.1 presents descriptive statistics for LS and FS (for descriptive purposes, untransformed data is presented) based on the learning curves for twins and their siblings. A significant age effect was found on LS (Δ-2LL = 4.02, df = 1, $p < 0.05$). For FS, no significant age effect was present (Δ-2LL = 0.00, df = 1, $p = 1.00$). Between the 9-year-old twins and their older siblings, the mean value of FS was significantly different (Δ-2LL = 20.55, df = 1, $p < 0.01$). The magnitude of the sex effect was the same in twins and siblings for both parameters (LS Δ-2LL = 0.54, df = 1, $p = 0.46$; FS, Δ-2LL = 0.41, df = 1, $p = 0.52$). Average LS was higher for girls than boys (Δ-2LL = 7.64, df = 1, $p < 0.01$). There was no significant sex effect on FS (Δ-2LL = 0.44, df = 1, $p = 0.51$).

Variances in twins were similar in all sex and zygosity groups for both LS and FS (LS, Δ-2LL = 4.24, df = 5, $p = 0.52$; FS, Δ-2LL = 8.21, df = 5, $p = 0.15$). Also the variance was similar for male and female siblings (LS; Δ-2LL = 0.35, df = 1, $p = 0.55$; FS, Δ-2LL = 0.25, df = 1, $p = 0.62$). Variances were similar in twins and sibling for LS (Δ-2LL = 0.00, df = 1, $p = 0.97$). For FS, the variance in twins was significantly larger than the variance in siblings (Δ-2LL = 7.33, df = 1, $p < 0.01$).

Table 4.1 Descriptives for Learning Speed and Forgetting Speed for 9-year-old twins and their older siblings.

	Learning Speed	Forgetting Speed
Mean Females	3.28	1.45 / 0.66 [a]
Male deviation	-0.27	n.s.
β age	0.23	n.s.
Variance	0.78	2.57 / 1.00 [a]

Means and variances on Forgetting Speed are given for untransformed data. Further analyses of Forgetting Speed were performed on transformed data; [a] Significant differences between twins and siblings on mean and variance (twins / sibling).

Table 4.2 presents twin and twin-sibling correlations and their 95% confidence intervals. The statistical power to test for sex effects on covariance structure was low and Table 4.2 gives MZ, DZ and twin-sib correlations pooled over sexes. The covariance for DZ twin pairs and DZ twin – sibling were similar for both LS (Δ-2LL = 0.99, df = 1, p = 0.32) and FS (Δ-2LL = 0.019, df = 1, p = 0.89).

Genetic modeling

An ADE model, with sex and age as covariates, was fitted to the data on LS. The results are given in Table 4.3a. A model with the variance due to D constrained at zero did not result in a significant deterioration of model fit (Δ-2LL = 1.19, df = 1, p = 0.14). Because a model with the variance due to A fixed at zero, resulted in a significant detoriation of model fit (Δ-2LL = 19.70, df = 1, p < 0.01), the AE model is considered to give the best description of the data (see Figure 4.3a). The total variance of LS was 0.79, where 0.34 (43%) was explained by additive genetic effects (A) and 0.45 (57%) explained by unique environment effects (E).

Because means and variances were different between twins and their older siblings for FS, they were allowed to differ in the genetic model for FS. The difference in variances between twins and sibs was modeled by including an additional variance component in twins (T). Total variance for twins was explained by the summation of A, D, E and T, while total variance in siblings remained the summation of A, D, and E. Table 4.3b contains the results of the model fitting on FS.

Table 4.2 Twin pair and twin sibling correlations and their 95% confidence intervals for Learning Speed and Forgetting Speed.

	Learning Speed [a]		Forgetting Speed		
		95% CI		95% CI	
	Correlation	(Lower – Upper)	Correlation	(Lower – Upper)	N
MZ twin pairs	0.49	0.27 – 0.64	0.41	0.07 – 0.63	44
DZ twin pairs	0.04	-0.23 – 0.30	0.07	-0.14 – 0.28	64
Twin – Sibling	0.18	0.03 – 0.32	0.07	-0.08 – 0.22	99

[a] Correlation for Learning Speed was corrected for age and sex

When the variance due to D was constrained at zero (i.e., AE(T) model), this did not result in a significantly worse model fit (Δ-2LL = 1.88, df = 1, p = 0.08). Setting the variance of A at zero, the model showed a significant detoriation in fit (Δ-2LL = 4.12, df = 1, p = 0.02). Therefore, the AE(T) model was considered as the best fitting model (Figure 3b), where 0.86 (20%, and 30% of total variance for twins and siblings, respectively) of the variance could be attributed to additive genetic effects and 1.99 (46% and 70% for twins and siblings, respectively) to unique environmental effects, and 1.51 (35%) for the extra variance in twins only (T).

Discussion

To our knowledge this is the first study on the genetic background of individual differences in the dynamics of verbal learning in a population-based sample of unselected young children. Non-linear learning curves were fitted to the data by estimating Learning Speed and Forgetting Speed (LS and FS), giving a more informative view on the dynamics during verbal learning.

Girls had higher LS than boys, while FS did not show any sex differences. LS increased with age, indicating that older children are able to add more new words to their phonological loop while performing the task. This finding is consistent with other studies, (Bishop et al., 1990;Forrester and Geffen, 1991;Gathercole, 1999;van den Burg and Kingma, 1999). There was no age effect on FS.

Twin correlations for both LS and FS were higher in MZ twins than in DZ twin pairs and twin-sibling pairs, suggesting genetic influences. LS heritability is estimated at 43%, and FS heritability between 20% and 30%. Because of the relatively small sample size it was not possible to explore sex differences on heritability. Although the differences in correlation between the MZ and the DZ twin pairs to some degree suggested dominance genetic influence (D), this was non-significant for both traits, possibly due to lack of power (Posthuma and Boomsma, 2000).

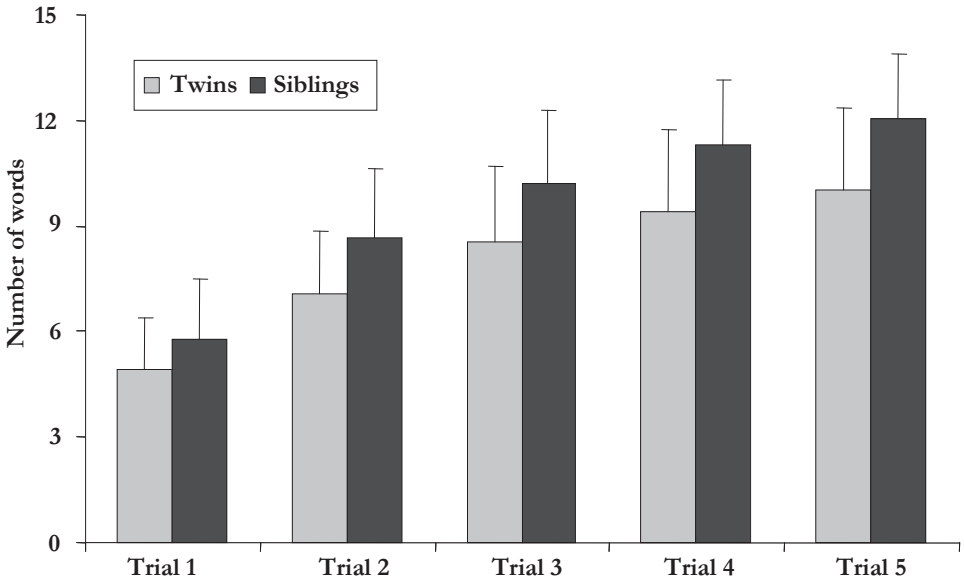

Figure 4.2 Mean (± s.d.) number of correctly recalled words on each trial of the AVLT by the 9-year-old twins and their older siblings.

Table 4.3 Results of the ADE model fit on Learning Speed (a) and on Forgetting Speed (b).

a Learning Speed

Model	A Estimated *(Lower/Upper)*	D Estimated *(Lower/Upper)*	E Estimated *(Lower/Upper)*	Heritability	-2LL	Df	p-value *
1. ADE	0.09 (0.00 – 0.45)	0.29 *(0.00 – 0.52)*	0.40 *(0.30 – 0.55)*	0.49	818.649	317	-
2. AE	**0.34** *(0.20 – 0.49)*	**-**	**0.45** *(0.35 – 0.58)*	**0.43**	819.843	318	0.14 (1)
3. E	0.79 *(0.69 – 0.90)*	-	-	-	839.543	319	< 0.01 (2)

b Forgetting Speed

Model	A Estimated *(Lower/Upper)*	D Estimated *(Lower/Upper)*	E Estimated *(Lower/Upper)*	T ** Estimated *(Lower/Upper)*	Heritability *(Twins/Siblings)*	-2LL	Df	p-value *
1. ADE(T) **	0.00 *(0.00 – 1.31)*	1.58 *(0.00 – 2.68)*	1.25 *(0.10 – 2.51)*	1.60 *(0.62 – 2.55)*	0.36 / 0.56	1343.780	317	-
2. AE(T)	**0.86** *(0.15 – 1.72)*	**-**	**1.99** *(1.15 – 2.95)*	**1.51** *(0.53 – 2.46)*	**0.20 / 0.30**	1345.663	318	0.08 (1)
3. E(T)	-	-	2.79 *(2.23 – 3.55)*	1.61 *(0.63 – 2.56)*	-	1349.786	319	0.02 (2)

Depicted are the amounts of variance (unstandardized) that can be attributed to Additive genetic (A), Dominance genetic (D), and Unique environment influences (E) and broad heritability estimations (sum of proportions of the total variance that can be attributed to A and D); * Superscript indicates references model that was used for the likelihood ratio tests; ** Amount of variance includes T component only in the twin group, presenting measurement error. Total variance for siblings does not include the T component.

There was a difference in both mean and variance of the FS parameter between the 9-year old twins and their older siblings, where the mean and variance were larger for the twins. Different explanations are possible for this finding. Differences in means between the two age groups are expected based on the growing literature on a developmental increase in verbal learning. The larger mean scores in twins were accompanied by larger variances. To explore if these larger variances could be attributed to a larger contribution of measurement error in the younger children, we estimated the covariance between DZ twins and between twins and siblings. If the reliable part of the measures is equally associated in these 2 groups, then this argues in favor of the measurement error hypothesis. As the covariances were similar, an extra variance component for twins was included in the genetic model for FS. A larger total variance in young children reduces heritability relative to the older group, because heritability is a ratio (genetic over total variance).

Heritability estimates can not be higher than the reliability of a test whereas reliability may be higher than heritability. Reliability of the FS and LS measures has to be explored in future research and therefore we do not know to what extent a low reliability might have influenced the present heritability estimates.

Swan and colleagues (1999) conducted a study in elderly twins, using principal component analysis on different variables derived from the CVLT. The component of verbal learning and memory, which might be of most interest, showed a heritability of 56%. However, this component included both STM and LTM measures, and is therefore difficult to compare to the presented LS and FS, which are representatives of immediate recall. Moreover, this study included elderly twin pairs, and it is known that heritability for general cognitive abilities increases across the life-span (Plomin et al., 2001; Bartels et al., 2002).

A similar paradigm, the non-word repetition task, has been used to explore the background of deficits in phonological loop functioning in healthy children (Byrne et al., 2006) and in children with specific language impairments (Gathercole et al., 1994). Deficits in this mechanism have been found to be of genetic origin in young children in the same age range as the present study (Bishop et al., 1996; Bishop et al., 1999; Bishop et al., 2006). Because of methodological differences, it is difficult to compare the heritability estimation between the specific language impairments studies and the present one. In addition to differences in experimental procedures of the two paradigms (i.e., non-word repetition task vs. AVLT paradigm with familiar words), different mechanism can underlie these two sorts of tasks. As a result, it is not unlikely that the magnitude of the genetic influences can differ, or maybe there are different sets of genes involved.

In conclusion, differences in verbal learning abilities are moderately heritable in healthy 9-year-old twin pairs and their older siblings.

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5

Heritability of verbal and performance intelligence in a pediatric longitudinal sample

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Abstract

The longitudinal stability of IQ is well-documented as is its increasing heritability with age. In a longitudinal twin study, we addressed the question to what extent heritability and stability differ for full scale (FSIQ), verbal (VIQ), and performance IQ (PIQ) in childhood (age 9-11 years), and early adolescence (age 12-14 years). Genetic and environmental influences and correlations over time were evaluated in an extended twin design, including Dutch twins and their siblings. Intelligence was measured by the Wechsler Intelligence Scale for children –Third version (WISC III). Heritability in childhood was 34% for FSIQ, 37% for VIQ, and 64% for PIQ, and increased up to 65%, 51%, and 72% in early adolescence. The influence of common environment decreased between childhood and early adolescence from explaining 43% of the phenotypic variance for FSIQ to 18% and from 42% for VIQ to 26%. For PIQ common environmental influences did not play a role, either in childhood or in early adolescence. The stability in FSIQ and VIQ across the 3-year interval (r_p) was 0.72 for both measures and was explained by genetic and common environmental correlations across time (FSIQ, $r_g = 0.96$, $r_c = 1.0$; VIQ, $r_g = 0.78$, $r_c = 1.0$). Stability of PIQ ($r_p = 0.56$) was lower and was explained by genetic influences ($r_g = 0.90$). These results confirm the robust findings of increased heritability of general cognitive abilities during the transition from childhood to adolescence. Interestingly, results for PIQ differ from those for FSIQ and VIQ, in that no significant contribution of environment shared by siblings from the same family was detected.

Introduction

General cognitive ability, or intelligence, is one of the most studied domains in the fields of psychology and behaviour genetics. Twin and adoption studies have explored the genetic and environmental influences on general cognitive abilities at different ages. An intriguing aspect of the etiology of general cognitive ability is the increase in genetic influences with increasing age, while common environment influences decrease (Davis et al., 2009; Deary et al., 2009; Haworth et al., 2010). In early childhood, genetic influences explain around 20-30% of the total variance of general cognitive ability. In middle childhood the relative importance of genetic influences increases up to around 40-50% (Bartels et al., 2002; Bishop et al., 2003; Davis et al., 2008). This increase in the relative influence of genetic factors continues and results in heritability estimates of 70% in young adolescence (Bartels et al., 2002), and 70-80% in adulthood (Posthuma et al., 2001; Bartels et al., 2002; Rijsdijk et al., 2002).

Psychometric IQ is remarkably stable across the lifespan (Deary et al., 2000; Bartels et al., 2002; Livingston et al., 2003; Hoekstra et al., 2007; Lyons et al., 2009). Longitudinal twin studies have generally found that genetic influences explain this stability (Boomsma and van Baal, 1998; Bartels et al., 2002; Davis et al., 2008; Davis et al., 2009; Lyons et al., 2009), that is, variation in IQ is explained by the same genetic factors at different ages, although in childhood there also is a contribution of shared environment (Boomsma and van Baal, 1998; Bartels et al., 2002; Davis et al., 2009) to stability.

General cognitive abilities are often assessed by extracting a first unrotated principal component from a series of test batteries (the g-factor), or by an intelligence quotient measure from a standardized intelligence test (i.e., full scale IQ; FSIQ). Usually these measures combine verbal and non-verbal tests. When verbal (VIQ) and non-verbal (performance IQ; PIQ) were analyzed separately in a longitudinal twin study of 5 to 18 year olds, some differences in genetic architecture emerged (Hoekstra et al., 2007). An increase in heritability was found for both scales. For VIQ, 28% of the variance was explained by common environment at the age of 5 years and this decreased to 6% at age 12. However, common environmental influences did not contribute to the variance of PIQ at any age. Stability in PIQ was entirely explained by genetic influences, while stability in VIQ was influenced by both genetic and common environmental factors (Hoekstra et al., 2007).

In the present longitudinal study 9-year-old twin pairs and their full siblings were assessed on general cognitive ability by standardized IQ tests. When the twins were 12 years old, they and their siblings returned at the follow-up. With this extended, longitudinal twin design an increase in power to detect sources of variance due to genetic and common environment influences was realized (Posthuma and Boomsma, 2000). In addition it allowed an examination of the causes of stability in full scale IQ, verbal and performance IQ.

Methods

Subjects

Participants were recruited from the Netherlands Twin Register (NTR; Boomsma et al., 2006). They took part in an ongoing longitudinal study into the development of cognition and brain maturation (van Leeuwen et al., 2008; Peper et al., 2008). Families were invited for participation around the 9th birthday of the twins. Exclusion criteria consisted of chronic use of medication, any known major medical or psychiatric history, or participation in special education. In total 112 families were included and one older sibling with a maximum age of 14 years was also invited to participate (N = 103). Twins and sibs were invited for a follow-up study around the 12th birthday of the twins. In total 89 families participated at follow-up, including 83 siblings. Table 5.1 provides their mean age and SD.

Zygosity of same-sex twin pairs was determined by DNA polymorphisms. The twin sample at baseline and follow-up consisted of 23/20 monozygotic male twin pairs (MZM), 25/20 monozygotic female twin pairs (MZF), 23/17 dizygotic male twin pairs (DZM), 21/17 dizygotic female twin pairs (DZF), and 20/15 dizygotic twin pairs of opposite sex (DOS).

At baseline there were 46 siblings who were close to the 9-year-old twins in age (mean age was 10.9 years, with a range of 9.9 up to 11.5 years, 20 girls and 26 boys). Their data were analyzed with the baseline data of the twins. In total 69 siblings were close in age to the 12-year-old twins (mean age was 12.8 years, with a range of 11.5 up to 14.0 years, 38 girls and 31 boys). These sibling data had either been collected during the first visit to the lab (N = 50), or at follow-up (N = 19).

Written informed consent was obtained from all subjects and their parents and the study was approved by the Dutch Central Committee on Research involving Human Subjects (CCMO). Parents were financially compensated for travel expenses and the children received a small gift each.

Measures

Participants were individually tested in separate rooms by experienced test administrators. At baseline the full version of the Wechsler Intelligence Scale for children –Third version (WISC-III; Wechsler et al., 2002) was used, including six verbal (information, similarities, arithmetic, vocabulary, comprehension, and digit span), and six nonverbal subtests (picture completion, coding, picture concepts, block design, picture assembly, and symbol search). At follow-up a shortened version of the WISC-III was administered, including four verbal subtests (similarities, arithmetic, vocabulary, and digit span), and two nonverbal subtests (picture completion, and block design). Test scores were corrected for the different number of subtests for verbal and nonverbal separately. For full scale, these corrected scores were summed, giving equal weight of verbal and nonverbal subtests to full scale performance. Raw scores were standardized according the age of the child at moment of testing, based on a population sample of same-aged subjects in the Netherlands (Wechsler et al., 2002), giving full scale intelligence quotient (FSIQ), as well as a verbal (VIQ), and nonverbal quotient (PIQ).

Genetic analyses

Monozygotic (MZ) twins are genetically identical and share (nearly) 100% of their genetic material, while dizygotic (DZ) twins and full siblings share on average 50% of their segregating genes. By comparing the MZ, DZ and twin-sibling covariance structures for a univariate or longitudinal phenotype, one can estimate the relative influences of genes and environment on phenotypic variation and on covariation among phenotypes. Additive genetic factors (A) represent the influences on the phenotype of multiple alleles at different loci on the genome that act additively. The proportion of variance in a trait that can be attributed to genetic factors is termed heritability. Common environmental influences (C) include all environmental factors that make twins and siblings who grow up in the same family resemble each other. Environmental factors not shared with other family members are referred to as unique environmental influences (E), and also include measurement error (Falconer and Mackay, 1996).

All data analyses were carried out with structural equation modelling (SEM) in the software package Mx (Neale et al., 2006). All available data were analyzed, i.e. regardless whether subjects participated once or twice in the study. Parameters were estimated by full-information maximum likelihood. Tests of significance of parameters were carried out by comparing the model fits of a model including that parameter to a model in which the parameter estimate was constrained at zero. The goodness of fit of different models was evaluated by comparing differences in log-likelihood.

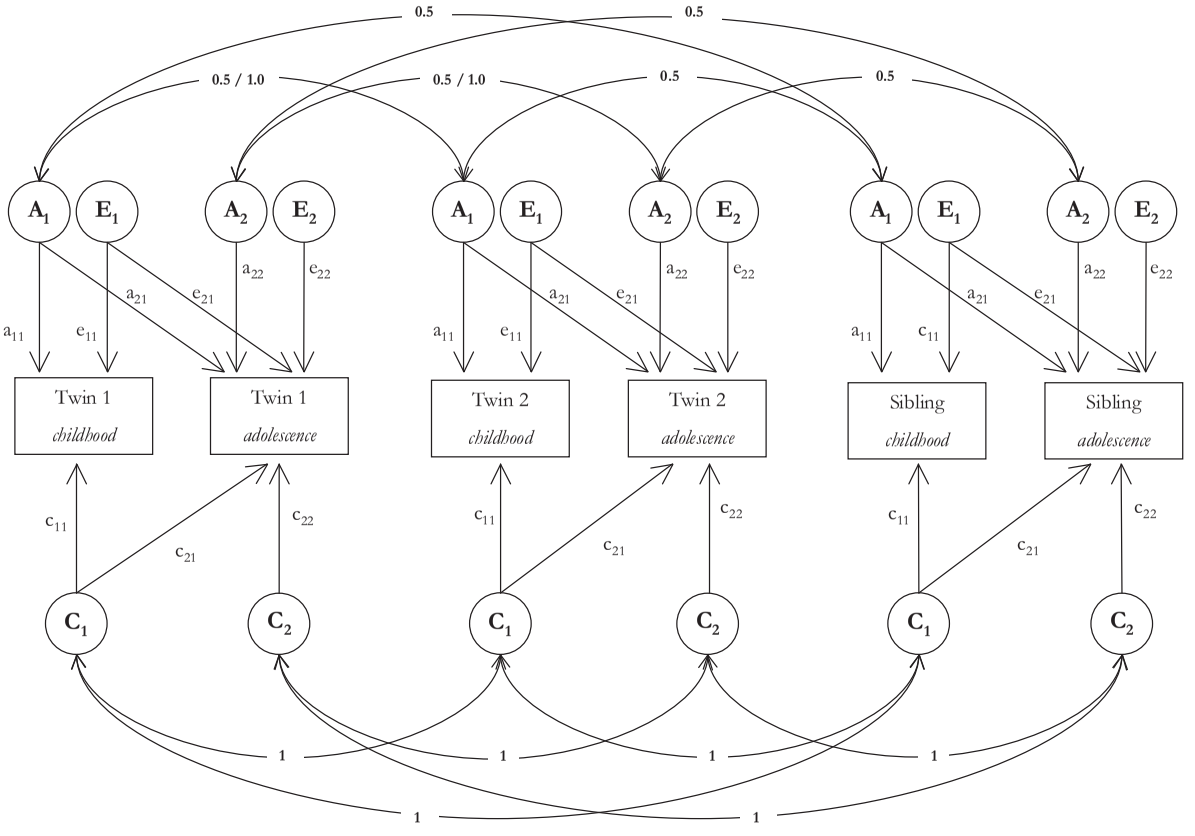

Figure 5.1 Path diagram representing the longitudinal genetic model fitted to the intelligence scores in childhood and early adolescence. Squares represent the measured variables (i.e. intelligence score), and the circles represent latent, unobserved factors. Double headed arrows represent correlations between the latent factors. The influence of the first set of latent factors on intelligence in childhood is represented by the path coefficients (one-headed arrows) a_{11}, c_{11}, and e_{11}, and in early adolescence by a_{21}, c_{21} and e_{21}. The second set of latent factors influence intelligence in early adolescence only, and is represented by the path coefficients a_{22}, c_{22} and e_{22}. Following path tracing rules; Var ($IQ_{childhood}$) = $(a_{11}^2) + (c_{11}^2) + (e_{11}^2)$, and Var ($IQ_{adolescence}$) = $(a_{21}^2 + a_{21}^2) + (c_{21}^2 + c_{22}^2) + (e_{21}^2 + e_{22}^2)$. In MZ twins the genetic factors are correlated 1.0 within twin pairs and 0.5 for DZ and siblings. In MZ, DZ and twin-siblings, the common environmental factors are correlated 1.0, and the unique environmental factors are uncorrelated.

Twice the difference between log likelihoods is chi-squared distributed with degrees of freedom (df) equal to the difference in the number of parameters estimated in the two models.

First, phenotypic correlations over time, MZ, DZ and twin-sibling correlations and cross-twin cross-time correlations were estimated for FSIQ, VIQ and PIQ. Equality of means and variances for twins and siblings and of DZ and twin-sibling correlations were tested.

Secondly, a longitudinal ACE model was fitted to the data (see Figure 5.1). The latent A, C and E factors in this model are standardized to have zero mean and unit variance. Path coefficients a_{11}, c_{11}, and e_{11} represent the influences of A_1, C_1, and E_1 on IQ in childhood. Path coefficients a_{21}, c_{21}, and e_{21} represent the influences of A_1, C_1, and E_1 on IQ in early adolescence. The path coefficients a_{22}, c_{22}, and e_{22} represent the influences of A_2, C_2, and E_2 on IQ in early adolescence (i.e., the part that is not influenced by A_1, C_1 or E_1). The total variance of IQ in childhood is given by the sum of the squares of a_{11}, c_{11}, and e_{11} ($Vp= a_{11}^2 + c_{11}^2 + e_{11}^2$). Heritability in childhood is given by $a_{11}^2 / (a_{11}^2 + c_{11}^2 + e_{11}^2)$. The total variance of IQ in early adolescence is given by the sum of the squares of a_{21}, a_{22}, c_{21}, c_{22}, e_{21}, and e_{22} ($Vp= (a_{21}^2 + a_{22}^2) + (c_{21}^2 + c_{22}^2) + (e_{21}^2 + e_{22}^2)$). As a result, the heritability in early adolescence is given by $(a_{21}^2 + a_{22}^2) / (a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2)$.

The covariance between IQ in childhood and IQ in early adolescence is derived from multiplying the path coefficients that define the association of childhood and early adolescence. The genetic covariance is given by ($a_{11} * a_{21}$), the common environmental covariance by ($c_{11} * c_{21}$), and the unique environmental covariance by ($e_{11} * e_{21}$). The total covariance is a summation: $(a_{11} * a_{21}) + (c_{11} * c_{21}) + (e_{11} * e_{21})$. The extent to which genetic influences in childhood and early adolescence overlap can be calculated as the genetic correlation $r_g = (a_{11} * a_{21}) / \sqrt{(a_{11}^2 * (a_{21}^2 + a_{22}^2))}$. In a similar way the common environmental and unique environmental correlations can be obtained. All correlations were tested to establish if they significantly contribute to the stability of intelligence measures across age by constraining a_{21}, c_{21} , or e_{21} at zero. In addition, all genetic and environmental correlations over time were tested whether they were significant different from 1.00, by constraining the path of a_{22}, c_{22}, and e_{22} at zero (implicating complete overlap of factors between the ages). As the sample is relatively small and sex differences in genetic architecture of IQ based measures have not or very seldom been reported (e.g., Haworth et al., 2010) we pooled the data for boys and girls.

Results

Mean age at testing and IQ scores for twins and siblings are given in Table 5.1. Full scale (FSIQ), verbal (VIQ), and performance IQ (PIQ) were normally distributed in all groups. Mean IQ scores were higher for siblings than for twins in both childhood and early adolescence for FSIQ ($\chi^2 = 18.58$, df = 1, $p < 0.01$), VIQ ($\chi^2 = 19.47$, df = 1, $p < 0.01$) and PIQ ($\chi^2 = 6.96$, df = 1, $p = 0.01$). For all IQ measures, variances in twins did not differ by birth order or zygosity. For VIQ, a scalar was included in the model to account for a larger variance in siblings ($\chi^2 = 6.20$, df = 2, $p = 0.04$).

Table 5.2 gives the phenotypic correlations across time, MZ and DZ twin, and twin-sibling correlations for FSIQ, VIQ and PIQ. Phenotypic correlations over time (r_p) were high for FSIQ ($r_p = 0.72$) as well as for VIQ ($r_p = 0.72$). PIQ showed a somewhat lower phenotypic correlation over time ($r_p = 0.56$). For all three IQ measures, MZ correlations were higher than DZ and twin-sibling correlations, indicating genetic influences. For FSIQ and PIQ, MZ correlations increased with age, while for VIQ, the MZ correlations remained approximately similar. The higher DZ correlations for VIQ imply that common environment is of importance for both age groups. DZ correlations were not different from the twin-sibling correlations in childhood (FSIQ, $\chi^2 = 0.56$, df = 1, $p = 0.45$; VIQ, $\chi^2 = 0.20$, df = 1, $p = 0.65$; PIQ, $\chi^2 = 3.03$, df = 1, $p = 0.08$), or in early adolescence (FSIQ, $\chi^2 = 0.43$, df = 1, $p = 0.51$; VIQ, $\chi^2 = 3.21$, df = 1, $p = 0.07$; PIQ, $\chi^2 = 0.68$, df = 1, $p = 0.41$).

Table 5.1 Mean (SD) of age at testing, full scale (FSIQ), verbal (VIQ), and performance (PIQ) intelligence scores for all subjects in childhood and early adolescence.

	Childhood		Adolescence	
	Twins *(N=224)*	**Siblings** *(N=46)*	**Twins** *(N = 177)*	**Siblings** *(N=69)*
Age at test (years)	9.1 (0.1)	10.9 (0.4)	12.1 (0.3)	12.8 (0.9)
FSIQ	99.9 (13.5)	105.7 (16.4)	100.3 (14.1)	106.5 (16.3)
VIQ	99.6 (14.5)	106.2 (17.7)	102.3 (12.5)	106.6 (15.8)
PIQ	100.1 (12.3)	103.5 (14.5)	98.3 (17.6)	106.7 (15.6)

Table 5.2 Phenotypic (r_p) and twin correlations, with their 95% confidence intervals of full scale (FSIQ), verbal (VIQ) and performance (PIQ) intelligence scores in childhood and early adolescence.

		Childhood			Adolescence		
	r_p	**rMZ**	**rDZ**	**rTwin-sibling**	**rMZ**	**rDZ**	**rTwin-sibling**
FSIQ	.72	.75	.54	.60	.81	.55	.47
	(.64 - .79)	(.63 - .84)	(.34 - .68)	(.45 - .72)	(.69 - .88)	(.34 - .70)	(.29 - .61)
VIQ	.72	.80	.62	.58	.76	.65	.44
	(.63 - .79)	(.69 - .87)	(.44 - .74)	(.41 - .70)	(.62 - .85)	(.45 - .78)	(.26 - .59)
PIQ	.56	.61	.23	.45	.74	.27	.38
	(.45 - .65)	(.43 - .64)	(-.02 - 0.45)	(.28 - .59)	(.58 - .84)	(.00 - .49)	(.19 - .54)

Parameters estimates from the longitudinal ACE models are given in Table 5.3. Each of the path coefficients was tested for significance. The model fitting statistics of the full ACE model and the submodels are given in Table 5.4.

The contributions of genetic, common environment and unique environmental influences to the stability of IQ measures between childhood and adolescence were tested for significance by constraining a_{21}, c_{21}, and e_{21} at zero. The genetic covariance was significant for FSIQ ($\chi^2 = 18.41$, $df = 1$, $p < 0.01$), VIQ ($\chi^2 = 10.72$, $df = 1$, $p = < 0.01$), and PIQ ($\chi^2 = 19.36$, $df = 1$, $p < 0.01$). Common environmental influences were contributing to stability over time for FSIQ ($\chi^2 = 7.75$, $df = 1$, $p = < 0.01$), and VIQ ($\chi^2 = 9.65$, $df = 1$, $p = < 0.01$), but not for PIQ ($\chi^2 = 2.07$, $df = 1$, $p = 0.08$). Unique environmental influences did not contribute to the stability of any of the IQ scales, indicating that these influences were not transmitted over time. Table 5.5 gives the proportion of variance that could be attributed to genetic, common and unique environmental influences on all three IQ scales in childhood and early adolescence. Genetic, common and unique environmental correlations are also displayed in Table 5.5.

Both the unstandardized genetic variance and the heritability increased for all IQ scales. Heritability of FSIQ increased from 34% in childhood to 65% in early adolescence. Common environmental influences on the other hand, decreased with age for FSIQ, namely from 43% in childhood down to 18% in early adolescence. For VIQ a similar pattern of genetic and environmental influences was observed. Heritability increased from 37% in childhood up to 51% in adolescence, while the contribution of common environmental factors decreased from 42% down to 26%. For PIQ, heritability increased from 46% up to 72%, and in contrast to the other IQ scales, common environment influences did not reach significance for PIQ in both age groups. When common environment was excluded from the model ($\chi^2 = 1.21$, df = 3, p = 0.75), heritability was 64% in childhood and 72% in early adolescence (Table 5.5).

Genetic correlations across time were 0.96 for FSIQ, 0.78 VIQ, and 0.90 for PIQ and all genetic correlations were found to be not significantly different from 1.00, because the path of a_{22} could be constrained at zero without a significant worse fit of the data. Common environmental correlations across time were 1.00 for both FSIQ and VIQ.

Discussion

Genetic and environmental influences on the stability of full scale (FSIQ), verbal (VIQ) and nonverbal (PIQ) intelligence scores in children (9-11 years) and in young adolescents (12-14 years) were explored in a sample of twins and siblings. The main finding was that the stability of FSIQ and VIQ was explained by genetic and to a smaller extent by common environmental influences, while stability of PIQ was completely explained by genetic influences during this important developmental period of the transition from childhood to early adolescence.

The influence of genes and common environment on the stability of FSIQ over time is in line with previous longitudinal studies in this age group (Boomsma and van Baal, 1998; Bartels et al., 2002; Bishop et al., 2003; Spinath et al., 2003; Davis et al., 2008; Davis et al., 2009). In addition to stable genetic and environmental influences some studies also observed age-specific genetic and common environmental influences (Bishop et al., 2003; Davis et al., 2008; Davis et al., 2009). In longitudinal studies intelligence measures often are based on different age appropriate test instruments. This potentially may introduce age-specific influences caused by the different test instruments used at different ages. In our study IQ measures were based on the WISC-III at both test occasions.

Table 5.3 Unstandardized parameter estimates for additive genetic (*a*), common environmental (*c*), and unique environment (*e*) path coefficients from the longitudinal model.

		a_{11}, a_{21} and a_{22}		c_{11}, c_{21} and c_{22}		e_{11}, e_{21} and e_{22}	
		childhood	**adolescence**	**childhood**	**adolescence**	**childhood**	**adolescence**
FSIQ	**childhood**	8.15		9.16		6.78	
	adolescence	11.53	3.55	6.25	0.00	0.42	6.21
VIQ	**childhood**	8.97		9.58		6.68	
	adolescence	6.99	5.52	6.33	-0.01	1.30	5.99
PIQ	**childhood**	8.51		5.12		7.72	
	adolescence	14.40	0.00	1.92	0.00	-1.72	8.70

Table 5.4 Model fit results for the longitudinal ACE model and the nested submodels for full scale (FSIQ), verbal (VIQ), and performance (PIQ) IQ. Model fit is given by -2 *log-likelihood (-2LL), and the degrees of freedom (df). All nested models are compared to the full ACE model.

	FSIQ				VIQ				PIQ			
Model	**-2LL**	*df*	χ^2	*p*	**-2LL**	*df*	χ^2	*p*	**-2LL**	*df*	χ^2	*p*
Full ACE longitudinal model	3919.68	503			3917.14	501			4062.40	503		
1. No genetic covariance (a_{21})	3938.09	504	18.41	**< .01**	3927.87	502	10.72	**< .01**	4081.77	504	19.36	**<.01**
2. No common environmental covariance (c_{21})	3927.43	504	7.75	**< .01**	3926.79	502	9.65	**< .01**	4062.67	504	0.27	0.30
3. No unique environmental covariance (e_{21})	3919.90	504	0.23	0.32	3919.63	502	2.49	0.06	4064.48	504	2.07	0.08
4. No genetic influence through a_{11}	3938.09	504	18.41	**< .01**	3928.35	502	11.21	**< .01**	4081.77	504	19.36	**< .01**
5. No genetic influence through a_{22}	3919.77	504	0.09	0.38	3919.27	502	2.13	0.07	4062.40	504	0.00	1.00
6. No common environmental influence through c_{11}	3930.01	504	10.33	**< .01**	3927.98	502	10.84	**< .01**	4063.61	504	1.21	0.14
7. No common environmental influence through c_{22}	3919.68	504	0.00	1.00	3917.14	502	0.00	1.00	4062.40	504	0.00	1.00

For verbal and nonverbal cognitive abilities, a different pattern of results between the two domains emerged. Stability of VIQ was influenced by both genetic and common environmental factors, while stability of PIQ was explained by genetic influences only. The number of longitudinal studies that separate the verbal and non-verbal cognitive abilities, using the VIQ and PIQ scales is limited. Similar results were observed in an independent longitudinal twin study (Hoekstra et al., 2007). In that study a larger number of ages (5, 7, 10, 12 and 18 year) were included and this enabled the testing of a transmission model. In this type of model, influences from earlier to later ages and, innovation terms unique for each age can be tested (Hoekstra et al., 2007). This study and the present study both contribute to the finding that during childhood and early adolescence verbal and nonverbal cognitive domains develop in different ways (Hoekstra et al., 2007).

When looking at the heritability estimates in childhood and early adolescence, an increase in heritability was observed for all IQ scales, which was due an increase in genetic variance. Heritability of FSIQ increased from 34% in childhood to 65% in early adolescence. Common environmental influences on FSIQ decreased in importance from 43% in childhood down to 18% in early adolescence. Heritability of VIQ increased from 37% in childhood to 51% in early adolescence. Common environmental influences on VIQ decreased from 42% in childhood down to 26% in early adolescence. Heritability of PIQ was 64% in childhood and increased up to 72% in early adolescence. There was no contribution of common environmental influences on PIQ in childhood or in early adolescence. This pattern of increasing genetic and decreasing common environmental influences with age are in line with other large studies (Bartels et al., 2002; Bishop et al., 2003; Hoekstra et al., 2007; Davis et al., 2009; Haworth et al., 2010).

However, an increase in the influences of genetic factors as seen for IQ is not the general rule for childhood behavioural traits. For example, heritability of externalizing problem behaviour was generally stable across the ages 3 up to 12, while a slight decrease in heritability was observed for internalizing behaviours across this age range (Bartels et al., 2004). A decrease in heritability by age was also observed in studies exploring anxious and depression symptoms, and withdrawn behaviour in childhood (Boomsma et al., 2005; Hoekstra et al., 2008). During adolescence heritability on depression and anxious symptoms and withdrawn behaviour were found to remain around 50% (Lamb et al., 2010). For attention problems, a trait which is associated with IQ (Polderman et al., 2006), heritability is uniformly high between ages 3 to 12 years (Rietveld et al., 2004). Between ages 13 and 25, an increase in heritability was observed in a large meta-analysis of externalizing behaviour, anxiety and depression symptoms (Bergen et al., 2007).

The increase in the heritability of intelligence may be the result of several processes. Genetic amplification has been previously suggested (DeFries et al., 1987), and this is what was observed in the present study. Also, as children grow older they are more likely to select or maybe even partly create their own environment, driven by their genetic disposition, resulting in an increased expression of their genetic potential (Plomin et al., 1977). Simultaneously, common environmental influences that are present in childhood diminish with increasing age. The increase of genetic and decrease of common environmental influences on IQ could be a result of children becoming more independent from their familial environmental and parental influences (Scarr and Mccartney, 1983). We now show that this is mainly driven by the verbal counterpart of intelligence, and not by nonverbal abilities.

Children undergo considerable improvements in their cognitive abilities throughout childhood and adolescence. Differences between children in verbal intelligence are explained not only by genetic differences but apparently also by the common environment. For example, maternal education, other parental influences, neighbourhood characteristics, and social economic status all tend to be associated with intelligence, verbal ability and reading skills in childhood and adolescents (Leventhal and Brooks-Gunn, 2000), which are all factors shared by children from the same home.

An additional explanation for a contribution in twin studies of common environment is assortative mating. For intelligence it has been found that spouses resemble each other in IQ scores. When resemblance of the parents is caused by phenotypic assortment, this can induce genetic similarity between the parents, which in turn affects the genetic similarities between siblings and increases resemblance in DZ twins and siblings. As a consequence the heritability can be underestimated and the estimates of common environmental influence inflated (Cavalli-Sforza and Bodmer, 1971). For IQ based on the Raven test, phenotypic assortment was found in the parents of the participating offspring who were tested at the baseline assessment of the present sample (van Leeuwen et al., 2008).

The increase in genetic variance found in all IQ scales holds valuable information for the fields of molecular genetics and of neuroscience, studying brain maturation and the associations of general cognitive abilities with brain changes during development. Despite the increase in heritability of intelligence up to relatively high estimates in adulthood, gene finding studies have not yet provided consistent results pointing to genetic variants that are associated with intelligence (Posthuma et al., 2005; Bochdanovits et al., 2009), although there are genetic variants that are associated with disorders where cognitive functioning is in some way affected (Flint, 1999; Deary et al., 2009). With respect to individual differences in intelligence in healthy samples, large genome wide association studies may very well provide more information on this in the near future. That individual differences in verbal and performance IQ each exhibit different etiology is an indication that the separation of these two intelligence domain can be informative. Although higher genetic influences are no guarantee for success in genome wide association studies (Manolio et al., 2009), the presence of genetic influences on a trait, and the contribution of the same genetic influences on stability of a trait over time is a preferred characteristic and reassuring when IQ scores across different ages are pooled for an increase in power in gene finding studies.

How the increase in genetic influences on intelligence is associated with developmental brain changes that occur around the period of transition form childhood to adolescence is still not fully understood. Measures for brain anatomy are under large genetic influences throughout early infancy (Gilmore et al., 2010;Smit et al., 2010), childhood and adolescence (Wallace et al., 2006; Schmitt et al., 2007; Peper et al., 2009; Smit et al., 2010; Yoon et al., 2010; Brouwer et al., 2010), and adulthood (Baare et al., 2001; Peper et al., 2007). While the genetic influences that explain individual differences in brain size seem to be generally stable at different ages, the human brain itself is a highly dynamic organ, and undergoes considerable developmental changes during development from infancy up to adulthood (Giedd et al., 1999). The genetic influences found for variation in brain anatomy showed an overlap with genetic influences on intelligence in adults (Posthuma et al., 2002; Hulshoff Pol et al., 2006), and in childhood and adolescence (van Leeuwen et al., 2009; Betjemann et al., 2010; Wallace et al., 2010). The amount of brain changes in cortical thickness in adults is under genetic influences, and partly overlap with genetic influences on IQ (Brans et al., 2010). There are indications that the level of intelligence is associated with developmental trajectories of the human cortex during adolescence (Shaw et al., 2006).

The mechanisms behind the associations between brain anatomy and function, and the developmental brain changes with the level of general cognitive abilities are not fully understood. Recent research is also focussing on the efficiency of the brain, by means of network analyses. A higher level of functional connectivity was found to be associated with higher levels of IQ (van den Heuvel et al., 2009). Furthermore, there are indications that distinct associations with brain anatomy are present when verbal and nonverbal cognitive abilities are explored separately (Betjemann et al., 2010; Wallace et al., 2010). There is more research needed to understand the etiology behind the stability of IQ over time and the possible relationship with brain changes, especially in this period in development.

Table 5.5 Proportion of total variance (and 95% confidence interval) that can be attributed to genetic (A), common environment (C), and unique environmental influences (E) for full scale (FSIQ), verbal (VIQ), and performance (PIQ) IQ and genetic (r_g), common environmental (r_c), and unique environmental (r_e) correlations across time.

		Childhood			Adolescence					
	Model	**A**	**C**	**E**	**A**	**C**	**E**	r_g	r_c	r_e
FSIQ	ACE	.34	.43	.23	.65	.18	.17	.96	1.00	.07
		(.14 - .61)	(.18 - .61)	(.16 - .34)	(.43 - .82)	(.02 - .38)	(.11 - .28)	(.74 - 1.00)	(.69 - 1.00)	(-.20 - .34)
VIQ	ACE	.37	.42	.21	.51	.26	.24	.78	1.00	.21
		(.11 - .64)	(.18 - .63)	(.13 - .32)	(.25 - .72)	(.06 - .46)	(.15 - .37)	(.53 - 1.00)	(.71 - 1.00)	(-.05 - .46)
PIQ	ACE	.46	.17	.38	.72	.01	.27	1.00	1.00	-.19
		(.20 - .73)	(.00 - .39)	(.26 - .51)	(.43 - .82)	(.00 - .25)	(.18 - .41)	(.73 - 1.00)	(-1.00 - 1.00)	(-.42 - .09)
	AE	.64		.36	.72		.28	.90		-.19
		(.51 - .75)		(.25 - .49)	(.58 - .82)		(.18 - .42)	(.74 - 1.00)		(-.42 - .07)

There were some limitations of the extended twin design used in the present study. The mean IQ score of the siblings was higher compared to their own co-twins in both age groups. This could be partly explained by studies that report that birth order within a family was associated with higher IQ score (Zajonc and Sulloway, 2007; Boomsma et al., 2008). In the majority of the included families the participating sibling is the oldest child in the family. Furthermore, the phenotypic correlation of PIQ across time was relatively low. One reason could be the use of a shortened version of the subscales used to measure performance IQ on the second test occasion.

To summarize, our main finding is that stability in verbal IQ is influenced by genetic and common environmental influences, while stability of performance IQ is driven by genetic influences. These findings contribute to the existing literature that verbal and nonverbal domains have different developmental trajectories. Children undergo considerable changes in their environment, but also in brain anatomy and function during this period in life, and the rates of these changes are most likely linked together in a complex manner. How the developmental trajectories of cognitive abilities are related with brain development has to be explored further in more detail.

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6

Heritability of volumetric brain changes and height in children entering puberty

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I.L.C. van Soelen, R.M. Brouwer, G.C.M. van Baal, H.G. Schnack, J.S. Peper, L. Chen, R.S. Kahn, D.I. Boomsma*, H.E. Hulshoff Pol* (* Equal authorship). Accepted for publication in *Human Brain Mapping*.

Abstract

The human brain undergoes structural changes in children entering puberty, while simultaneously children increase in height. It is not known if brain changes are under genetic control, and whether they are related to genetic factors influencing the amount of overall increase in height. Twins underwent MRI brain scans at age 9 (N=190) and 12 (N=125). High heritability estimates were found at both ages for height and brain volumes (49% - 96%), and high genetic correlation between ages were observed (r_g > 0.89). With increasing age, whole brain (+1.1%), cerebellum (+4.2%), cerebral white matter (+5.1%), and lateral ventricle (+9.4%) volumes increased, and third ventricle (-4.0%) and cerebral gray matter (-1.6%) volumes decreased. Children increased on average 13.8 cm in height (9.9%). Genetic influences on individual difference in volumetric brain and height changes were estimated, both within and across traits. The same genetic factors influenced both cerebral (20% heritable) and cerebellar volumetric changes (45%). Thus, the extent to which changes in cerebral and cerebellar volumes are heritable in children entering puberty are due to the same genes that influence change in both structures. The increase in height was heritable (73%), and not associated with cerebral volumetric change, but positively associated with cerebellar volume change (r_p = 0.24). This association was explained by a genetic correlation (r_g = 0.48) between height and cerebellar change. Brain and body each expand at their own pace and through separate genetic pathways. There are distinct genetic processes acting on structural brain development, which can not be explained by genetic increase in height.

Introduction

The development of children entering puberty is characterized by considerable structural brain changes (Giedd et al., 1999), and a rapid increase in height (Tanner, 1962; Tanner & Whitehouse, 1976). From young childhood up to puberty and adulthood, complex, non-linear, and region specific structural changes occur in the human brain (Giedd et al., 1999). White matter tissue increases in volume, while gray matter tissue volume increases in young childhood and after peaking around puberty starts to decrease (Giedd et al., 1999; Sowell et al., 2002; Paus, 2005; Lenroot et al., 2007). These changes in structural brain development are of functional significance in healthy children (Shaw et al., 2006). Deviant brain developmental changes are associated with developmental brain disorders (Gogtay et al., 2002; Arango et al., 2008; Shaw et al., 2010; Courchesne et al., 2011; Wallace et al., 2010). The increase in the incidence of psychopathologies emerging during and right after puberty (Kessler et al., 2007; Paus et al., 2008) suggest a possible causal relationship with aberrant brain structure changes that occur in this period.

Genetic factors largely determine height in adulthood (Baare et al., 2001; Silventoinen et al., 2003), and in childhood (Silventoinen et al., 2007). This also holds for whole brain volumes which are to a high level heritable in adults (Baare et al., 2001; Peper et al., 2007; Kremen et al., 2010), as well as in children and adolescents (Wallace et al., 2006; Schmitt et al., 2007a; Schmitt et al., 2007b; Peper et al., 2009; Yoon et al., 2010). Moreover, we recently found that changes in brain structures were heritable in healthy adults (Brans et al., 2010) and in adults with schizophrenia (Brans et al., 2008). It is not known to what extent genetic factors influence brain changes during the period from childhood to adolescence, where probably different processes underlie structural brain changes compared to adulthood.

In the present study we address the question whether brain volume changes are a reflection of the strong increase in height, that occurs in children at the beginning of puberty (Tanner, 1962; Tanner & Whitehouse, 1976), or whether they reflect a different process, controlled by other genes. We studied the extent to which genes influence volumetric brain changes and increase in height in childhood and early adolescence. Furthermore, we explored whether the genetic influences acting on brain changes were overlapping with the genetic influences acting on increase in height in children entering puberty.

Materials and methods

Subjects

Twin families were recruited from the Netherlands Twin Register (NTR; Boomsma et al., 2006), and an epidemiologically representative sample of the Dutch population. The children were invited to participate in a large longitudinal twin study to explore the genetic and environmental influences on brain maturation (see for more details of the study; van Leeuwen et al., 2008; Peper et al., 2009). Exclusion criteria for participation included having a pacemaker, any metal materials in the head (including dental braces), chronic use of medication, a known major medical or psychiatric history, and participation in special education.

At baseline data on height were available for 218 twins, and 208 twins completed the structural scan procedure at the same day at the University Medical Center Utrecht (UMCU), The Netherlands. Response rate at follow-up was nearly 80%, resulting in 173 twins with available data on height. At follow-up, 136 twins completed the structural scan procedure at the UMCU. The major cause for missing scans at the follow-up was the higher prevalence of metal braces. Mean (s.d.) full scale IQ of the complete sample was 99.9 (13.5) at age 9, and 100.3 (14.1) at 12, illustrating that the sample was representative of the general population (van Soelen et al., 2011).

Secondary sexual characteristics of puberty, i.e. breast development in girls and penis development in boys, were determined by a trained researcher according Tanner criteria (Marshall & Tanner, 1969; 1970). At baseline, 6% of the boys and 18% of the girls showed first stages of puberty (i.e., Tanner stage ≥ 2). At follow-up, 56% of the boys and 68% of the girls showed development of secondary sexual characteristics, and within these groups the girls were more progressed in puberty stage than the boys (mean (s.d.) Tanner stage girls was 2.9 (1.1); boys was 2.1 (0.9)).

After exclusions based on scan quality, 190 scans at baseline and 125 scans at follow-up could be included for further image processing. Mean age at the moment of scan at baseline was 9.2 (s.d. = 0.1; range = 9.0 – 9.7) years, and at follow-up 12.1 (s.d. = 0.3; range = 11.7 - 13.1) years old. In total, 113 children had scans available at both measurements. Mean interval time between the two measurements was 2.9 (s.d. = 0.2; range = 2.5 - 3.5) years.

Zygosity of the same-sex twin pairs was determined based on DNA polymorphisms. At baseline the sample with available MRI and height data consisted of 82 (39 male / 43 female) MZ twins (38 complete twin pairs; 17 male / 21 female), 75 (38 male / 37 female) same-sex DZ twins (32 complete twin pairs; 16 male / 16 female), and 33 opposite-sex DZ twins (14 complete twin pairs). At follow-up the sample consisted of 56 (30 male / 26 female) MZ twins (23 complete twin pairs; 13 male / 10 female), 45 (23 male / 22 female) same-sex DZ twins (18 complete pairs; 8 male / 10 female), and 24 opposite-sex twins (10 complete pairs). Handedness was determined based on Edinburgh Handedness Inventory (Oldfield, 1971). Parents and the children themselves gave written informed consent to participate in the study.

Image acquisition

All structural magnetic resonance imaging (MRI) was performed on a 1.5-T Philips Achieva scanner on both measurements. To limit possible effects of scanner instability over time, the same scan parameters as well as image processing procedures were used at both baseline and follow-up. All children underwent a practice session in a dummy scanner to get familiarised with the scan procedure, small space and the sounds of the MRI machine (Durston et al., 2009). At both baseline and follow-up image-sequences of the whole head were acquired, including a short scout scan for immediate verification of optimal head positioning, and a clinical scan that was used for neurodiagnostic evaluation. A three-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head (256 × 256 matrix, TE = 4.6 ms, TR = 30 ms, flip angle = 30º, 160-180 contiguous slices; 1 × 1 × 1.2 mm^3 voxels, Field-of-View = 256 mm / 70%) was acquired for volumetric analysis. Additionally, a DTI-B0 (transverse; 15-64 directions; SENSE factor 2.5, b-factor 1000; flip angle 90 º; 60 slices of 2.5 mm; slice gap 0; 128 x 96 acquisition matrix; FOV = 240 mm; TE = 78 ms) and a MTI (transverse; MTC frequency offset 1100 Hz; 60 slices of 2.5 mm; slice gap 0;128 x 96 acquisition matrix; FOV 240 mm; flip angle 8 º ; TE = 4.5 ms; TR = 37.5 ms) were acquired on both baseline and follow-up (as previously described in Peper et al., 2008). At follow-up, a T2-weighted image was added to the scan protocol for optimization of image processing as described below (transverse, parallel imaging, SENSE factor 2, TE1 = 15 ms, TE2 = 80 ms, TR = 6000 ms, flip angle = 90 º , 120 slices of 1.6 mm, slice gap 0.0 mm, Field-of-view = 250 mm / 80%).

Image processing

All image processing steps were conducted at the UMCU. Scans were put into Talairach frame (no scaling), and corrected for inhomogeneities in the magnetic field (Sled et al., 1998). Quantitative assessment of intracranial volume (IC) of the first measurement was based on the

DTI-BT0 and MTI images as described earlier (Peper et al., 2008). The IC segments for follow-up were created from the baseline IC segments using nonlinearly transformations. The T1-weighted images of the baseline measurements were nonlinearly warped onto the follow-up measurement up to a scale of 1.2 mm full-width-at-half-maximum by a combination of nonlinear warpings with increasing precision (Collins et al., 1995). This transformation was subsequently applied to the baseline intracranial mask. When no IC segment was available from baseline, the T2- weighted image at follow-up was used to create an IC segment (N= 10). For one participant no scan data at baseline and no T2-weighted scan at follow-up were available, and therefore the same method as described for the baseline measurement was used (DTI-BT0 and MTR) to create an IC segment for the follow-up measurement. All IC segments at baseline and follow-up were checked and edited where necessary. Total brain, and gray and white matter were segmented using a partial volume segmentation method incorporating a non-uniform partial volume distribution (Brouwer et al., 2010). Cerebellar, lateral ventricles, and third ventricle volumes were assessed (Schnack et al., 2001), and these segments were visually checked and edited where necessary. For one individual lateral ventricles and third ventricle volumes could not be segmented reliably at baseline.

Genetic analyses

Monozygotic (MZ) twin pairs are genetically identical and share (nearly) 100% of their genetic material, while dizygotic (DZ) twin pairs and full siblings share on average 50% of their segregating genes. By comparing the MZ and DZ covariance structures on a specific phenotype, one can estimate the relative influences of genes and environment on variation of that phenotype (Boomsma et al., 2002). Additive genetic influences (A) represent the influences on the phenotype of multiple alleles at different loci on the genome that act additively. The proportion of the observed variance in a trait that can be attributed to genetic factors is termed heritability. Common environmental influences (C) include all environmental sources of variance that make twins who grow up within the same family resemble each other. Environmental influences that are unique to an individual and not shared with other family members are referred to as unique environmental influences (E), and also included measurement error (Falconer & Mackay, 1996).

If phenotypic variance is influenced by genetic factors, MZ twins will resemble each other more than the DZ twins. If MZ twin resemblance is twice as high as DZ twin resemblance, additive genetic influences are of importance. However, when DZ resemblance is higher than half MZ resemblance, both genetic and common environmental influences are contributing to the phenotypic variance. Finally, when DZ and MZ resemblance is equal, then only common environment can explain twin resemblance (Plomin et al., 2001; Boomsma et al., 2002).

Longitudinal genetic modelling

With longitudinal data it is possible to determine to what extent the covariance of a specific trait across time is due to correlated genetic and environmental effects. Differences in the cross-twin / cross-time correlation between MZ and DZ twin pairs contain information about the etiology of this association over time. Larger MZ cross-correlations between time points (twin 1 at baseline with twin 2 at follow-up and vice-versa) compared to the DZ cross-correlations indicate that there is overlap between genetic factors at baseline and follow-up.

A longitudinal ACE model was fitted to the data (see Figure 6.1). For detailed description of the path diagram see also van Soelen, et al., (2011). The covariance between a phenotype (i.e., brain volume) at baseline and at follow-up is derived from multiplying the path coefficients that define the association between baseline and follow-up on that specific brain volume. The genetic covariance is given by ($a_{11} * a_{21}$), the common environmental covariance by ($c_{11} * c_{21}$), and the unique environmental covariance by ($e_{11} * e_{21}$). The total covariance is a summation of these three covariances, namely ($a_{11} * a_{21}$) + ($c_{11} * c_{21}$) + ($e_{11} * e_{21}$). The extent to which genetic factors influence brain volume at both baseline and follow-up can be calculated as the genetic correlation, $r_g = (a_{11} * a_{21}) / \sqrt{(a_{11}^2 * (a_{21}^2 + a_{22}^2))}$. In a similar way the common environmental and unique environmental correlations can be obtained.

The longitudinal model included a calculation to gain insight into the contribution of genetic influences on individual differences in height or volumetric brain *changes* (see Figure I). The total variance on changes in height and brain volumes can be calculated by the following general formula; V change = $(a_{11}^2 + c_{11}^2 + e_{11}^2) + ((a_{21}^2 + a_{22}^2) + (c_{21}^2 + c_{22}^2) + (e_{21}^2 + e_{22}^2)) - (2 * (a_{11} * a_{21}) + (c_{11} * c_{21}) + (e_{11} * e_{21}))$. The contribution of genetic influences on changes in height and brain volumes can be derived by $(a_{11}^2) + (a_{21}^2 + a_{22}^2) - (2 * (a_{11} * a_{21}))$. In a similar way the contributions of common and unique environmental variance on changes in height and brain volumes can be calculated. Based on

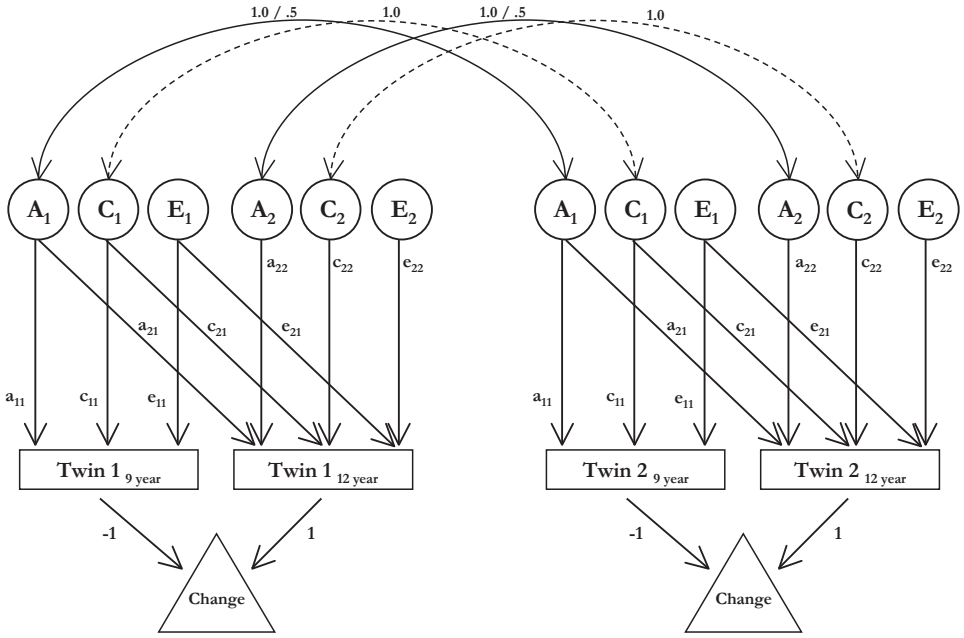

Figure 6.1 Path diagram representing the longitudinal genetic model fitted to the data collected at age 9 and 12. Squares represent the observed phenotype of interest (e.g, height or brain volumes) in twin 1 and twin 2, and the circles represent latent, unobserved factors. A_1, C_1, and E_1 influence the phenotype at age 9 and 12 years; A_2, C_2, and E_2 influence the phenotype at age 12, but not at age 9 years. Double headed arrows represent correlations between the genotypes of twins (1.0 for MZ and 0.5 for DZ pairs). The influence of the first set of latent factors on the phenotype at age 9 is represented by factor loadings (one headed arrows) a_{11}, c_{11}, e_{11}, and at age 12 by a_{21}, c_{21}, and e_{21}. The second set of latent factors influence the phenotype at age 12 only, and is represented by the path coefficients a_{22}, c_{22} and e_{22}. The triangle represents the developmental change for height or the different brain volumes.

these formulas, genetic influences on change in height or brain volumes can only be present when there is either a change in effect size of the same genetic factors, the presence of different genetic factors that exert their influence at the different ages, or a combination of the above described genetic mechanisms (de Geus et al., 2007).

Genetic influences on changes in height and brain volumes

For the brain volumes where significant heritability is found on volumetric change, it is of interest to explore to what extent the genetic and environmental influences are shared between different structures. In addition, it is of interest whether these genetic factors in turn show an overlap between the genetic influences acting on changes in height. Therefore, we performed additional genetic modelling on the change scores. Instead of studying cross-twin/cross-time correlations, as described above, we investigated cross-twin/cross-trait correlation, thereby modeling the covariance structure between two or more different phenotypes, i.e. change in height (cm), volumetric change in structure A and in structure B (ml). The genetic correlations that can be derived by calculations as illustrated above now describe the overlap of genetic influences acting on change in height and brain volumes, or the overlap of genetic influences on volumetric changes between different brain volumes.

Statistical analyses

Changes in height and brain volumes observed within subjects over time were tested for significance by using a paired t-test in SPSS, where the degrees of freedom (df) were adjusted for familial dependency. Genetic analyses were carried out using structural equation modelling (SEM) using the software package Mx (Neale et al., 2006). All available data were analyzed, i.e. regardless whether subjects participated once or twice in the study. Parameters were estimated by maximum likelihood. This was done for each trait separately. For brain volumes, data were corrected for sex, age at scanning and handedness at baseline and follow-up. For height, data were corrected for sex and age only.

In the first series of analyses, differences by birth order or zygosity in means and variances where tested. Twin correlations for MZ and DZ twin pairs were estimated for height and all brain volumes at both baseline and follow-up. In the second series of analyses, a longitudinal genetic model was fitted to the data (see Figure 6.1). Tests of significance of parameters were carried out by first estimating them and then constraining the estimates at zero (e.g., a_{11}, a_{21}, see Figure 6.1). The goodness of fit of different models was evaluated by comparing the difference in log-likelihood. The difference between -2-log likelihood is chi-squared distributed with the degrees of freedom (df) equal to the difference in the number of parameters estimated in two nested models.

Results

In Table I the means (s.d.) of age, height and the observed volumetric measures are given, as well as the means (s.d.) of changes in height and in brain volumes between baseline and follow-up. Height and all brain volumes were normally distributed at both assessments, with the exception of the lateral and third ventricle volumes. After log transformation, these volumes were normally distributed. Changes in height and brain volumes were all normally distributed.

Table 6.1 Mean (s.d.) of age at moment of scan (years), height (cm), brain volumes (ml) at baseline (N=190), follow-up (N=125) and the differences between baseline and follow-up on height and brain volumes (N=113) are given. Height and volumetric brain changes were calculated as percentage change compared to baseline. Height and all brain volumes showed a significant within-subject volumetric change between baseline and follow-up (paired t-test), where p-values are corrected for familial dependence by adjusting the degrees of freedom.

	Baseline	**Follow-up**	**Changes over time**		
	Mean *(s.d.)*	**Mean** *(s.d.)*	**Mean** *(s.d.)*	**% change compared to baseline**	**p-value**
Age at scan	9.2 *(0.1)*	12.1 *(0.3)*	2.9 *(0.2)*		
Height *	138.8 *(5.3)*	152.3 *(7.1)*	13.8 *(3.6)*	9.9 %	< .01
Total brain	1332.8 *(114.3)*	1352.0 *(120.3)*	14.6 *(16.8)*	1.1 %	< .01
Cerebrum	1164.7 *(103.7)*	1182.0 *(110.4)*	12.4 *(15.1)*	1.1 %	< .01
Cerebrum GM	703.8 *(59.2)*	692.5 *(63.0)*	-11.4 *(18.4)*	-1.6 %	< .01
Cerebrum WM	460.9 *(52.7)*	489.5 *(56.7)*	23.8 *(18.2)*	5.1 %	< .01
Cerebellum	153.4 *(14.4)*	159.6 *(14.2)*	6.4 *(2.8)*	4.2 %	< .01
Cerebellum GM	107.7 *(10.8)*	109.6 *(10.0)*	2.7 *(5.5)*	2.5 %	< .01
Cerebellum WM	45.6 *(6.4)*	50.0 *(7.0)*	3.7 *(5.8)*	8.1 %	< .01
Lateral ventricle	9.6 *(7.0)* **	10.8 *(7.8)*	0.9 *(0.9)*	9.4 %	< .01
Third ventricle	0.65 *(0.30)*	0.65 *(0.30)*	-0.03 *(0.16)*	-4.0 %	< .05

GM = gray matter, WM = white matter. * Data on height were available from more children, total N baseline = 218, N follow-up = 173, N with two measurements = 173. ** For lateral ventricle volume at baseline is total number of scans is 189.

Between baseline and follow-up, children showed differences in height and brain volumes (Table 6.1). Height, total brain, cerebral and total cerebellar volumes all showed a within subject increase. Children increased on average 9.9% in height. Total brain and cerebrum showed an increase of 1.1% in volume. Cerebellar volume showed a larger increase of 4.2% compared to baseline. After separation of gray and white matter tissue in the cerebrum and cerebellum, increased cerebral white matter (5.1%), cerebellar white (8.1%) and gray matter (2.5%) volumes were observed, while cerebral gray matter showed a decrease in volume (-1.6%). A relatively large increase was observed for lateral ventricles (9.4%) and the third ventricle decreased in volume (-4.0%). In Figure 6.2 individual volume changes can be observed between the two assessments for total cerebral, total cerebellar, and cerebral white and gray matter volumes.

Height was not different for boys or girls at baseline (mean (s.d.) was 139.5 (5.6) cm in boys and 138.2 (4.8) cm in girls; $p = 0.35$) or at follow-up (mean (s.d.) was 152.1 (7.1) cm in boys and 152.6 (7.1) cm in girls; $p = 0.22$). The change in height was significantly different between boys and girls (mean change (s.d.) was 12.9 (3.1) cm in boys and 14.7 (3.9) cm in girls; $p < 0.01$). Boys had significantly larger brain volumes than girls ($p < 0.01$). There were no significant differences between boys and girls on volumetric brain changes between baseline and follow-up ($p > 0.12$ for all brain volumes).

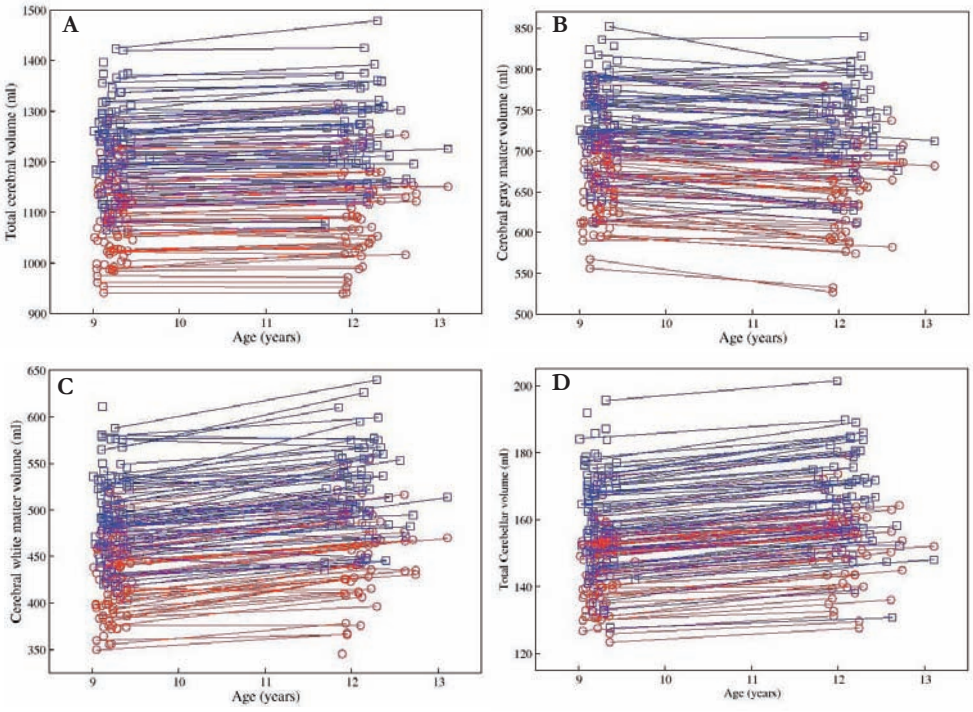

Figure 6.2 Absolute brain volume of total cerebral volume (A), cerebral gray matter (B), cerebral white matter (C), and total cerebellar volume (D) are given on all included subjects at baseline and follow-up. Longitudinal data points are connected, and boys are depicted in blue, and girls in red.

Genetic modelling

Variances did not differ between birth-order and zygosity groups for height and all brain volumes. Total variances at follow-up were significantly larger than total variances at baseline for height and all brain volumes, with the exception of cerebellar gray matter and lateral ventricle volume, where the variances at both ages were similar.

Within pair correlations for MZ and DZ twins and the 95% confidence intervals (CI) for height and all brain volumes at baseline and follow-up can be found in Table 6.2. MZ correlations were higher than DZ correlations at both ages, indicating genetic influences at baseline and follow-up. Twin correlations were of the same magnitude at both ages, with exception of cerebellar gray matter, where MZ correlations were lower at follow-up than at baseline.

A longitudinal genetic model was evaluated that included A, C, and E effects. For height, influence of common environment was not significant. Although lateral ventricles volume showed some suggestive influence of common environment at both baseline and follow-up, common environmental influences were found to be non-significant for all brain volumes. Therefore, we continued with a longitudinal AE model.

Table 6.3 and 6.4 present the unstandardized and standardized estimates of genetic (i.e., heritability) and environmental influences on height and the brain volumes. Overall, heritability estimates at baseline and follow-up were high for height and brain volumes (see Table 6.3). Somewhat lower heritability estimations were observed for the cerebellar white matter volume (i.e., 64% at baseline and 49% at follow-up).

For height and all brain volumes high genetic correlations over time were observed, indicating that individual variation in height and brain volumes at the ages of 9 and 12 were completely explained by the same genetic factors. Indeed, no significant contributions of age specific genetic factors at baseline or follow-up were observed.

Table 6.2 Monozygotic (MZ) and dizygotic (DZ) twin correlations with their 95% confidence intervals (CI) are displayed at baseline and follow-up separately for height and brain volumes.

	Baseline		Follow-up	
	r MZ *(95% CI)*	**r DZ** *(95% CI)*	**r MZ** *(95% CI)*	**r DZ** *(95% CI)*
Height	.94 *(.90 - .96)*	.56 *(.37 - .70)*	.93 *(.89 - .96)*	.46 *(.32 - .71)*
Total brain	.93 *(.88 - .96)*	.44 *(.17 - .64)*	.96 *(.93 – .98)*	.44 *(.17 - .57)*
Cerebrum	.93 *(.89 - .96)*	.43 *(.17 - .64)*	.96 *(.93 - .98)*	.45 *(.19 - .65)*
Cerebral GM	.88 *(.80 - .92)*	.46 *(.20 - .64)*	.91 *(.83 - .95)*	.43 *(.16 - .62)*
Cerebral WM	.89 *(.81 - .93)*	.43 *(.15 - .63)*	.88 *(.78 - .93)*	.46 *(.13 - .67)*
Cerebellum	.95 *(.90 – .97)*	.52 *(.28 - .69)*	.96 *(.93 - .98)*	.49 *(.22 - .67)*
Cerebellar GM	.92 *(.86 - .95)*	.38 *(.10 - .58)*	.75 *(.61 - .85)*	.37 *(.03 - .61)*
Cerebellar WM	.62 *(.41 - .75)*	.49 *(.21 - .68)*	.50 *(.14 - .72)*	.37 *(-.08 - .65)*
Lateral ventricles *	.81 *(.70 - .88)*	.55 *(.29 - .71)*	.78 *(.64 - .87)*	.52 *(.27 - .69)*
Third ventricle *	.57 *(.32 - .73)*	.29 *(-.12 - .53)*	.55 *(.31 - .72)*	.27 *(-.20 - .64)*

GM = gray matter, WM = white matter. Data on height and brain volumes were corrected for age at scanning, sex, and handedness (for brain volumes only).* Twin correlation for lateral ventricles and third ventricle volumes were estimated based on log-transformed data.

Table 6.3 The standardized (heritability) estimates within the longitudinal AE model are given at baseline, follow-up and of the amount of height and volumetric brain changes for each variable separately. Genetic correlation (r_g) is the extent of overlap between genetic influences at baseline and follow-up.

	Baseline		Follow-up		Change score		
	A	E	A	E	A	E	r_g
Height	**.93** *(.89 - .96)*	.07 *(.04 – 11)*	**.93** *(.88 - .95)*	.07 *(.05 - .12)*	**.73** *(.58 - .83)*	.27 *(.17 - .42)*	**.92** *(.88 - .95)*
Total brain	**.93** *(.89 - .96)*	.07 *(.04 - .11)*	**.96** *(.93 - .98)*	.04 *(.02 - .07)*	**.19** *(.07 - .47)*	.81 *(.53 - .93)*	**1.00** **
Cerebrum	**.93** *(.89 - .96)*	.07 *(.04 - .11)*	**.96** *(.93 - .98)*	.04 *(.02 - .07)*	**.20** *(.08 - .45)*	.80 *(.55 - .92)*	**1.00** **
Cerebral GM	**.88** *(.81 - .93)*	.12 *(.08 - .19)*	**.91** *(.84 - .95)*	.09 *(.05 – .15)*	.03 *(.00 - .49)*	.97 *(.51 – 1.00)*	**1.00** **
Cerebral WM	**.89** *(.82 - .93)*	.11 *(.07 - .18)*	**.89** *(.81 - .94)*	.11 *(.06 - .19)*	.18 *(.00 - .45)*	.82 *(.55 – 1.00)*	**.98** *(.95 – 1.00)*
Cerebellum	**.95** *(.91 – .97)*	.05 *(.03 - .09)*	**.95** *(.92 - .97)*	.05 *(.03 – .08)*	**.45** *(.12 - .68)*	.55 *(.32 - .88)*	**.99** *(.98 – 1.00)*
Cerebellar GM	**.93** *(.88 - .95)*	.07 *(.05 - .12)*	**.80** *(.69 - .87)*	.20 *(.13 - .31)*	.03 *(.00 - .28)*	.97 *(.72 – 1.00)*	**1.00** **
Cerebellar WM	**.64** *(.46 - .76)*	.36 *(.24 - .54)*	**.49** *(.18 - .70)*	.51 *(.30 - .82)*	.13 *(.00 - .41)*	.87 *(.59 – 1.00)*	**.89** *(.61 – 1.00)*
Lateral ventricles *	**.80** *(.68 - .87)*	.20 *(.13 - .32)*	**.75** *(.60 - .84)*	.25 *(.16 - .40)*	.29 *(.00 - .68)*	.71 *(.32 – 1.00)*	**1.00** **
Third ventricle *	**.61** *(.42 - .76)*	.39 *(.24 - .58)*	**.59** *(.40 - .73)*	.41 *(.27 - .60)*	.02 *(.00 - .25)*	.98 *(.75 – 1.00)*	**1.00** **

GM = gray matter; WM = white matter; Data on height and brain volumes were corrected for age at scanning, sex, and handedness (for brain volumes only); Bold numbers differed significantly from zero (p> .05); * Variance components for lateral ventricles and third ventricle volumes were estimated on log-transformed data; ** correlation estimated at its upper bound.

Table 6.4 The unstandardized variance estimates within the longitudinal AE model are given at baseline, follow-up and of the amount of height and volumetric brain changes for each variable separately.

	Baseline		Follow-up		Change score	
	A	E	A	E	A	E
Height	23.99	1.70	44.00	3.43	7.98	3.00
Total brain	7060.92	525.79	8273.09	345.05	47.99	207.06
Cerebrum	5955.57	437.01	6986.01	288.41	41.09	166.17
Cerebral GM	2093.66	282.78	2404.71	223.82	10.77	308.24
Cerebral WM	1573.41	194.05	1841.95	218.49	62.71	277.49
Cerebellum	138.17	8.17	165.36	7.50	5.09	4.34
Cerebellar GM	92.94	8.73	79.49	19.78	0.53	28.13
Cerebellar WM	19.56	10.20	17.92	18.78	4.69	29.67
Lateral ventricles *	3.86	0.98	3.60	1.22	0.04	0.09
Third ventricle *	2.44	1.53	3.06	2.13	0.04	1.43

GM = gray matter, WM = white matter. Data on height and brain volumes were corrected for age at scanning, sex, and handedness (for brain volumes only). * Variance components for lateral ventricles and third ventricle volumes were estimated on log-transformed data.

Significant heritability on change in height (73%) was found. Significant heritability on volumetric brain changes were found for total brain (19%), total cerebrum (20%), and total cerebellum (45%). For all these variables, this was a result of increases in genetic variances, which were found to be significant for height, total brain, total cerebral and cerebellar volume ($p < 0.01$). Because no contribution of age-specific genetic factors at baseline or follow-up were found ($r_g > 0.90$), the heritability on changes in height, total brain, cerebral, and cerebellar volumes were caused by amplification of genetic factors already present at baseline (see Table 6.4).

To explore to what extent the genetic and environmental influences acting on developmental changes are shared between changes in height and brain volumes, we performed additional multivariate genetic analyses on these change scores. Longitudinal data were available for height in 173 children and for brain volumes in 113 children. Table 6.5 contains the phenotypic, genetic and unique environmental correlations between changes in height, cerebral and cerebellar volumes. The change in height was not associated with change in cerebral volume ($r_p = 0.09$, $p = 0.33$). Because of previous observed sex differences in change in height, correlations were explored in boys and girls separately, given a correlation of -0.07 in boys ($p = 0.59$) and 0.25 in girls ($p = 0.06$). Phenotypic correlation on the complete sample between change in height and cerebellar volume was 0.24 ($p = 0.01$), where a correlation of 0.06 was observed in boys ($p = 0.64$), and 0.37 in girls ($p < 0.01$). Correlation between change in cerebral and cerebellar volume was 0.49 ($p < 0.01$), where a correlation of 0.49 was observed in boys ($p < 0.01$), and 0.58 in girls ($p < 0.01$). Although both boys and girls show correlation in the same directions, girls seem to reflect higher phenotypic correlations compared to boys between changes in height and brain volumes, but also on changes between cerebral and cerebellar volumes.

The etiology of these associations were explored in more detail. The genetic influences acting on the amount of cerebral growth and the genetic influences acting on the amount of cerebellar growth showed an almost complete overlap ($r_g = 0.88$, $p < 0.05$). Interestingly, there was also a significant unique environmental correlation ($r_e = 0.34$, $p < 0.05$). The correlation between cerebellar growth and increase in height was a result of significant genetic correlation ($r_g = 0.48$, $p < 0.05$) and non-significant environmental correlation ($r_e = -0.06$, $p = 0.76$).

Table 6.5 Results of the multivariate genetic model including the change in height (cm), and cerebral and cerebellar growth (ml). Phenotypic correlation (r_p) between height and cerebral and cerebellar volume changes are given, as well as the extent of overlap in genetic (r_g), and unique environmental influences (r_e) acting on height, cerebral and cerebellar growth with their 95% confidence intervals.

	r_p*	r_g	r_e
Change in height and cerebral volume	0.09 (-0.10 – 0.27)	0.39 (-0.18 – 1.00)	-0.13 (-0.45 – 0.22)
Change in height and cerebellar volume	0.24 (0.06 – 0.41)	0.48 (0.10 – 1.00)	-0.05 (-0.41 – 0.32)
Change in cerebral and cerebellar volume	0.49 (0.33 – 0.62)	0.88 (0.02 – 1.00)	0.34 (0.06 – 0.59)

Total number of twins with available data on changes in height was 173 and cerebral and cerebellar volume changes were 113; *Phenotypic correlations (95% CI) for male and female twins were between change in height and cerebral volume was -0.07 (-0.30 – 0.18) in boys and 0.25 (-0.01 – 0.48) in girls, change in height and cerebellar volume was 0.06 (-0.19 – 0.30) in boys and 0.37 (0.12 – 0.57) in girls, change in cerebellar and cerebral volume was 0.49 (0.28 – 0.66) in boys and 0.58 (0.38 – 0.73) in girls.

Discussion

This is the first longitudinal study investigating genetic and environmental contributions to volumetric brain changes in children. The main findings of this study are three-fold: Firstly, height and brain volumes are highly heritable traits at both ages 9 and 12 years (up to > 90%). The stability that is seen for individual differences in these traits across age is explained by overlapping genetic factors (r_g > 0.89), i.e., the same genes influence individual differences in brain volumes at ages 9 and 12. Secondly, in children entering puberty there is a 1.1% overall brain volume increase over this three year period that is most pronounced in the cerebellum which increased by 4.2%. Interestingly, while it encompasses only 12 percent of the total brain, the change in cerebellar volume was responsible for one third of whole brain volume increase. Individual differences in volumetric brain changes were partly under genetic control (change in cerebral volume was 20% heritable and in cerebellar volume 45%). The association between changes in cerebellar and cerebral volumes (r_p = 0.49) was driven by shared genetic influences and, to a smaller extent, by shared unique environmental influences. Thirdly, the change in height was highly heritable (73%), but was influenced by other genetic factors than the genetic factors implicated in changes in cerebral volume. What little was shared between height and cerebellar growth (r_p = 0.24), could be attributed partly to shared genetic influences (r_g = 0.48). We can conclude that we not only find developmental brain changes in this three year interval, as reported earlier over larger age spans in children and adolescents (Giedd et al., 1999), we also find these changes to be heritable. Furthermore, these changes in cerebral volume are not shared with the increase that seen in height, while the change in cerebellar volume is partly (genetically) correlated with changes in height.

We show that brain size is heritable in children. For example, total cerebral volume is highly heritable at age 9 (93%) and this heritability increases at age 12 (96%). We showed now for the first time that changes in height and brain volumes are heritable and that these genetic influences on the changes are caused by amplification of genetic factors already present at baseline. This genetic factor is also implicated in changes of both cerebral and cerebellar volumes and thus seems to represent a general structural brain developmental factor. This genetic factor appears to influence brain tissue specifically, because we found little overlap with other changes in children entering adolescence, as represented here by their increasing height. This increase in height was a poor predictor of changes in cerebral volume, and was correlated to a smaller extent with changes in cerebral volume between 9 and 12. This suggests that cerebral brain development follows its own genetic pathway, which involves, at least during the age range of 9-12 years, the same genes as those implicated in overall head size. Change in cerebellar volume is partly correlated with change in height.

The finding of a general genetic factor acting on structural brain changes during childhood and early adolescence does not exclude that other genetic factors may be implicated in overall head size and volumetric changes over time later in life. In adult twins who were on average 30 years of age (ranging from 19 - 55 years) genetic influences on structural brain change differed from that implicated in brain structure (Brans et al., 2010). Possibly, the genetic mechanisms influencing volumetric changes differ in adolescence from those implicated in adulthood. For example, the genetic influences that occur during childhood brain development may be more intertwined with processes related to brain maturation compared to structural brain changes in adulthood, where they might represent a different process. Future longitudinal research, following the same individuals from childhood into adulthood, is the only way to provide the answers to these questions.

We also find that cerebral and cerebellum white matter increased in volume, while cerebral gray matter volume decreased between 9 and 12 years. Other studies exploring developing changes in children from young childhood up to adulthood reported that gray matter volume started to decrease around the age of 10-12 (Giedd et al., 1999; Giedd et al., 2006; Lenroot et al., 2007). This

decrease in gray matter volume is not observed in the cerebellum, which can be explained by the fact that the cerebellum is a structure that shows a relatively late maturation process (Tiemeier et al., 2010). We found that changes in cerebellar volume were relatively large compared to changes in cerebral volume. Indeed, there is more suggestive evidence that the cerebellum volume increases considerably around this age period (Tiemeier et al., 2010). The biological processes underlying these volumetric changes in gray and white matter around puberty are only scarcely understood. One of the hypotheses of the biological mechanism underlying gray matter loss is the process of synaptic pruning (Huttenlocher & Dabholkar, 1997). Another interpretation involves increased myelination of axons, which causes white matter volume increase and gray matter volume decrease, i.e., white matter encroachement (Paus et al., 2008; Gogtay & Thompson, 2010).

Heritability estimates for brain volumes were very high at both ages 9 and 12, reaching up to 96%. Furthermore, there was a large overlap between the genetic factors acting on brain volumes at ages 9 and 12. The extent to which the individual differences in volumetric changes are reliable seem to be driven by genetic influences, as was observed for total brain, total cerebrum and cerebellum. No significant genetic influences on changes for white and gray matter volumes were found. Although we observed some increase in genetic variance on gray and white matter volumes from age 9 to 12, and high genetic correlations over time, this was not accompanied by significant heritability estimates for gray or white matter volumetric changes. This may be a function of statistical power and samples size. Although the sample is large for imaging standards, it could be that even larger samples are needed to detect a subtle heritability on volumetric changes of gray and white matter. Findings of the variation in gray and white matter changes being mainly environmental need not to be entirely surprising. There are several studies that illustrate structural brain changes as a result of environmental factors (e.g., Draganski & May, 2008). For example, structural changes were observed after training on a complex visuo-motor task (Draganski et al., 2004), whole body balancing task (Taubert et al., 2010), or after a period of extensive learning (Draganski et al., 2006). The effects of these specific training paradigms cannot directly be compared to the normal development in a sample of unselected children, who are exposed randomly to a wide variety of environmental factors of every day life, but still serve to illustrate that environmental factors can be of importance in structural brain changes.

Heritability estimates for brain volumes were at least as high at age 12 as at age 9 years, as also reported earlier (Peper et al., 2009). The heritability estimates for total brain, cerebrum and gray and white matter are comparable to those of other cross-sectional pediatric twin studies (Wallace et al., 2006). The only exception seem to be cerebellum volume, which was found to be highly heritable in the present study, while a lower heritability was reported in an earlier study. However, in this earlier study (Wallace et al., 2006) children between the ages 4 through 19 years were included which makes heritability interpretation per age group challenging. To date, only one other twin study explored a twin sample with a narrow age range. In 8 year old twins, lower but significant heritabilities for brain volumes were reported (Yoon et al., 2010). For example, in this study heritability estimates of 71% for total brain volumes, 65% for total gray matter, and 80% for total white matter volume were found (Yoon et al., 2010).

No significant influences of common environmental influences shared by family members were seen on global volumetric measures. This is similar to findings over the whole range of imaging studies, which all acknowledge that common environmental effects do not reach significance. Thus, it seems that familiar influences are of little or no importance on overall brain development in children entering puberty.

The heritability estimates for both height and brain volumes are likely to reflect the involvement of many different genes. There are many biological processes that all can have some effect on how tall a person will be or how large the brain volume will become. For example, specific polymorphisms of the Brain Derived Neurotrophic Factor (BDNF) were found to be associated with prefrontal

cortex and hippocampal volume in healthy individuals (Pezawas et al., 2004). Another example is the Epsilon 4 allele of the Apolipoprotein gene (APOE), which is associated with Alzheimer disease. This genetic variant was associated with an altered developmental brain trajectory in children (Shaw et al., 2007). Finding the actual genes, or the specific alleles that are associated with variation in a trait of interest is a challenging job, not only for height in adults (Allen et al., 2010) and brain measures in healthy individuals (Thompson et al., 2010), but as well for psychiatric disorders (van Haren et al., 2008; Manolio et al., 2009).

When interpreting the results of this study several limitations have to be taken into consideration. When investigating a sample consisting of twins only can raise questions about the justification to generalize the results to the overall population, consisting of mostly singletons. Brain volumes of singletons and twins were previously found to be comparable in childhood (Ordaz et al., 2009), and in adulthood (Hulshoff Pol et al., 2002). Thus, we may assume that these results on brain volume changes in children can also be generalized to the singleton population.

In the present study, data of boys and girls were pooled when exploring changes in height and the association with brain growth. It is known that girls enter puberty at an earlier age than boys (Mul et al., 2001), which is accompanied with a growth spurt at the average age of 11 in girls and 14 years in boys (Tanner et al., 1966; Tanner & Whitehouse, 1976). It is possible that because girls are earlier with the start of puberty they find themselves in a slightly different phase of body or brain development than boys, as illustrated by the more advanced Tanner stage of puberty in the girls than the boys in the current sample. We did not observe sex differences in the amount of brain growth. However, we did observe a larger increase in height for the girls. In girls there also was a slightly higher association between change in height and brain volumes, but confidence intervals around the phenotypic correlation are large and overlapping between boys and girls. Higher correlations between change in height and brain volumes in girls gives an interesting perspective of possible sex differences in developmental stages of body and brain, but these results should be interpreted with caution, considering the sample size. Mean height (and changes) were always corrected for sex to limit the possible confounding effects of sex to a minimum.

We report on global brain volume changes only. However, it may be expected that there are considerable focal changes in brain structure present in these children as based on earlier findings on age related changes on cortical thickness (Gogtay et al., 2004). Genes exert specific influences on cortical thickening and thinning in adults (Brans et al., 2010), and on white matter density at age 9 years (Peper et al., 2009).

Because of the birth cohort approach we can only present longitudinal changes on a limited age-range, in this case between 9 and 12 years, and inferences regarding changes in more advanced stages of puberty and young adulthood await further longitudinal follow up in this and other samples. However, the strong point of this study is the focus on children entering puberty, which represents a unique period in the transition from childhood into adulthood, for both body and brain.

We have shown that changes in brain volumes from childhood to early adolescents, particularly prominent in the cerebellum, are heritable. There are shared genes and shared unique environmental influences responsible for the association between changes in both cerebral and cerebellar volumes. Simultaneously, children grow considerable in height in the same period. The amount of increase in height was heritable, but these genetic influences did not show an overlap with the genetic influences implicated in cerebral, and to a smaller extent with cerebellar volumetric increases. Therefore, we can conclude that there are distinct genetic processes acting on brain development, which could not be explained by simply the increase in height during the same period.

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7

Genetic influences on thinning of the cortical cortex during development

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I.L.C. van Soelen, R.M. Brouwer, G.C.M. van Baal, H.G. Schnack, J.S. Peper, D.L. Collins, A.C. Evans, R.S. Kahn, D.I. Boomsma*, H.E. Hulshoff Pol* (*Equal authorship). *In revision.*

Abstract

During development from childhood to adulthood the human brain undergoes considerable thinning of the cerebral cortex. Whether developmental cortical thinning is influenced by genes and if independent genetic factors influence different parts of the cortex is not known. Magnetic resonance brain imaging was done in twins at age 9 (N = 190) and 12 years (N = 125; 113 repeated measures) to assess genetic influences on development in cortical thinning. We find that considerable thinning of the cortex between the ages 9 and 12 (locally up to 0.24 mm; 10%; on average 0.05 mm; 1.5%), particularly in the frontal poles, and orbitofrontal, paracentral, and occipital cortices. Importantly, cortical thinning was found to be highly heritable in children (locally up to h^2=79%), and the degree of genetic influence differs for the various areas of the brain. For example, one factor drives both left inferior frontal (Broca's area), and left parietal (Wernicke's area) thinning; a second factor influences left anterior paracentral (sensory-motor) thinning. Other factors drive cortical thinning in the frontal poles: one of decreasing influence over time, and another independent genetic factor at age 12 in left and right frontal poles. Thus, thinning of the cerebral cortex is heritable in children between the age 9 and 12. Furthermore, different genetic factors are responsible for variation in cortical thickness at ages 9 and 12, with independent genetic factors acting on cortical thickness across time and between various brain areas.

Introduction

Our understanding of human brain development has expanded exponentially over the past decade. The non-invasive nature of magnetic resonance imaging (MRI) has allowed measurements of changes in brain structure in healthy children and adults. Using MRI, it has become evident that the human cerebral cortex undergoes considerable developmental changes during childhood and adolescence. After extensive growth of the brain in gray and white matter volume during early childhood, gray matter volume starts to decrease around puberty (Giedd et al., 1999). This is due to 0a considerable thinning of the cortex that occurs in children continuing throughout adolescence (Gogtay et al., 2004; Sowell et al., 2002), and beyond in adulthood (Brans et al., 2010). Timing of cortical thinning differs between areas. During development in childhood, thinning of primary cortical areas precedes that of secondary cortical areas (Gogtay et al., 2004). Currently it is not known to what extent genetic and environmental influences drive these brain *changes* during the crucial period of transition from childhood to adulthood. Brain volume (Peper et al., 2009) and cortical thickness (Yoon et al., 2010) are heritable traits in children and heritability estimates increase with age (Lenroot et al., 2009; Wallace et al., 2006). Recently, cortical thickness change was found to be under the influence of genes in young adults. Interestingly, these genetic influences on cortical changes were independent of those implicated in absolute cortical thickness (Brans et al., 2010). However, whether one single genetic factor influences cortical thinning during adolescence, or in contrast whether multiple (independent) genetic factors affect different brain areas is not known. We hypothesized that 1) cortical thinning between ages 9 and 12 is heritable; 2) during brain development the influence of genes on cortical thickness increases. In addition new genetic variance acting on cortical thickness emerges between the ages of 9 and 12; 3) genes exert their influence on the development of the cerebral cortex in a region-specific manner.

Material and methods

Subjects

Twin pairs were recruited from the Netherlands Twin Register (NTR; Boomsma et al., 2006) around their 9th birthday (van Leeuwen et al., 2008; Peper et al., 2008). Exclusion criteria for participation included having a pacemaker, any metal materials in the head (including dental braces), the chronic use of medication, a known major medical or psychiatric history, and participation in special education.

At age 9 a total of 208 twins completed the scan protocol (coming from 106 families). Due to movement artefacts, 8 MZ and 10 DZ twins could not be included for image processing of the scans. As a result, a total of 190 twins were included in the analyses at age 9, consisting of 82 MZ twins (38 complete twin pairs), 75 same-sex DZ twins (32 complete twin pairs), and 33 opposite-sex DZ twins (14 complete twin pairs). Mean age (s.d.) of twins at baseline was 9.2 (0.1) with a range from 9.0 up to 9.7 years old (Table 7.1).

Nearly 80% of the families returned for the follow-up at age 12, and 136 twins completed the scan procedures. The lower number of children that actually underwent scanning at follow-up was mainly due to the increase in prevalence of metal braces at age 12, which is common around this age. After exclusion due to movement artefacts, 125 scans at age 12 (excluding 7 MZ and 4 DZ twins) were included in the analyses. As a result, the second measurement consisted of 56 MZ twins (23 complete twin pairs), 45 same-sex DZ twins (18 complete pairs), and 24 opposite-sex twins (10 complete pairs).

Table 7.1 Sample descriptives of monozygotic (MZ) and dizygotic (DZ) same sex and opposite sex twin pairs at age 9, age 12, and of the twins that were included with complete datasets at both measurements.

	Baseline	Follow-up	Both measurements
Total number of twins *(male/ female)*	190 (91 / 99)	125 (66 / 59)	113 (60 / 53)
Mean age at scanning in years *(s.d.)*	9.2 (0.1)	12.1 (0.3)	2.9 (0.2) *
Handedness *(right / non-right)*	158 / 32	105 / 20	95 / 18
MZ twins *(number of complete pairs)*	82 (38)	56 (23)	51 (25)
DZ same-sex twins *(N complete pairs)*	75 (32)	45 (18)	40 (19)
DZ opposite-sex twins *(N complete pairs)*	33 (14)	24 (10)	22 (11)

* Mean time interval in years is given between scans at baseline and follow-up.

Mean age (s.d.) at follow-up was 12.1 (0.3) years, ranging from 11.7 up to 13.1 years old. At baseline and follow-up, 113 children had MRI data at both measurements available (42 complete twin pairs). Mean interval time was 2.9 years (0.2), and the range was 2.5 up to 3.5 years. Zygosity of the same-sex twin pairs was determined based on DNA polymorphisms. Handedness was determined based on Edinburgh Handedness Inventory (Oldfield, 1971).

Parents and the children themselves gave written informed consent to participate in the study. The study was approved by the Central Committee on Research involving Human Subjects (CCMO) of the Netherlands and experiments were in accordance with the Declaration of Helsinki.

Image acquisition

To limit possible effects of scanner instability over time, the same scanner parameters as well image processing procedures were applied at both measurements. All structural magnetic resonance imaging (MRI) was performed on a 1.5-T Philips Achieva scanner at both ages 9 and 12. Scanning and the subsequent image processing took place at the University Medical Center Utrecht, The Netherlands. All children underwent a practicing session in a dummy scanner in advance to get familiarised with the scan procedure, small space and the sounds of the MRI machine (Durston et al., 2009). At both measurements image-sequences of the whole head were acquired, including a short scout scan for immediate verification of optimal head positioning, and a clinical scan that was used for neurodiagnostic evaluation. Three-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head (256 × 256 matrix, TE = 4.6 ms, TR = 30 ms, flip angle = 30°, 160-180 contiguous slices; 1 × 1 × 1.2 mm^3 voxels, Field-of-View = 256 mm / 70%) was acquired for volumetric analysis. Additionally, a DTI-B0 (Diffusion Tensor Imaging; transverse; 15-64 directions; SENSE factor 2.5, b-factor 1000; flip angle 90°; 60 slices of 2.5 mm; slice gap 0; 128 x 96 acquisition matrix; FOV 240 mm; TE=60-88 ms) and a MTR (Magnetic Transfer Ratio; transverse; MTC frequency offset 1100 Hz; 60 slices of 2.5 mm; slice gap 0; 128 x 96 acquisition matrix; FOV 240 mm; flip angle 8°; TE=4.5 ms; TR=37.5 ms) were acquired at both age 9 and 12 (as previously described by Peper et al., 2008). At follow-up only, a T2-weighted

image was added to the scan protocol (transverse, parallel imaging, SENSE factor 2, TE1 = 15 ms, TE2 = 80 ms, TR = 6000 ms, flip angle = 90°, 120 slices of 1.6 mm, slicegap 0.0 mm, Field-of-view = 250 mm / 80%) for optimisation of image processing as described below.

Image Processing

Scans were put into Talairach frame (no scaling), and corrected for inhomogeneities in the magnetic field (Sled et al., 1998). Quantitative assessment of intracranial volume (IC) at age 9 was based on the DTI-BT0 and MTR images as described earlier (Peper et al., 2008). For the segmentation of IC volume at age 12, the T1-weighted image was individually warped non-linearly to their T1-weighted image at age 9 (when available). The IC segments at age 12 were created from the IC segments at age 9 using this nonlinearly transformation. When no IC segment was available at age 9, the T2- weighted image at age 12 was used to create an IC segment (N= 10). For one participant, the same method as described for 9 year old assessment was used (DTI-BT0 and MTR) to create an IC segment for the follow-up at age 12. All IC segments at age 9 and age 12 were checked and edited where necessary. Cerebral spinal fluid, gray and white matter were segmented using a partial volume segmentation method incorporating a non-uniform partial volume distribution (Brouwer et al., 2010).

Cortical thickness was computed using a custom version of the CLASP algorithm, designed at the McConnell Brain Imaging Centre, Montreal (Kim et al., 2005;Lerch et al., 2008), which started from the gray and white matter segments created by our own algorithm as described above (Brans et al., 2010). A 3D surface was fitted to the white matter/gray matter interface, which created the inner surface of the cortex and than expanded out to fit the gray matter/cerebrospinal fluid interface, thereby creating the outer cortical surface (Kim et al., 2005). Cortical thickness was estimated by taking the distance between the two surfaces on each vertex (40962 vertices per hemisphere), followed by smoothing across the surface using a 20 mm full-width at half-maximum (FWHM) surface-based blurring kernel. This method of blurring simultaneously improves the chances of detecting population differences and also follows the curvature of the surface to preserve any anatomical boundaries within the surface. The surfaces of the subjects were registered to an average surface (International Consortium for Brain Mapping; Lyttelton et al., 2007), allowing comparison of cortical thickness locally between and within subjects over time. In addition, mean cortical thickness was calculated for each person at both ages 9 and 12 (no smoothing).

Study design

A longitudinal twin design was applied. Twin studies are widely used to quantify to what extent genetic and environmental factors influence individual differences in observed traits, by comparing the similarities within MZ and DZ twin pairs. A larger correlation between members of MZ pairs compared to DZ pairs indicates genetic influences on a certain trait, such as, cortical thickness. The longitudinal follow-up of twins of the same age allows for estimation of cross-twin / cross-time correlations, i.e. cortical thickness age 9 in one twin correlates with its co-twin's values at age 12. Larger MZ cross-correlations than DZ cross-correlations indicate that there is an association between genetic factors at age 9 and 12. If MZ cross-correlations are not larger than DZ cross-correlations there is no such overlap in genetic factors over time. Similarly, cross-twin / cross-area correlations can elucidate whether different genetic factors are implicated across the cerebral cortex.

Genetic modelling

Because MZ twins share almost all their genetic material, and DZ twin pairs share approximately half of their segregating genes with each other, twin data allow for decomposition of the observed (or phenotypic) variance into genetic and environmental factors. Additive genetic influences (A) are effects on the phenotype of multiple alleles at different loci on the genome that act additively. Common environmental influences (C) represent sources of variance that twins and siblings share within the same family. Unique environmental influences (E) are sources of variance that are not shared with other family members (Boomsma et al., 2002;Falconer and Mackay, 1996). A, C, and E can be considered to be latent factors, standardized to have unit variance. By definition, the correlation between the latent genetic factor A of twin 1 and twin 2 is 1.0 for MZ and 0.5 for DZ twin pairs. The correlation between common environmental influences is 1.0 for both MZ and DZ pairs. Unique environmental influences (E) are by definition uncorrelated in all twin pairs, and also include measurement error. This correlation structure among latent factors identifies the model.

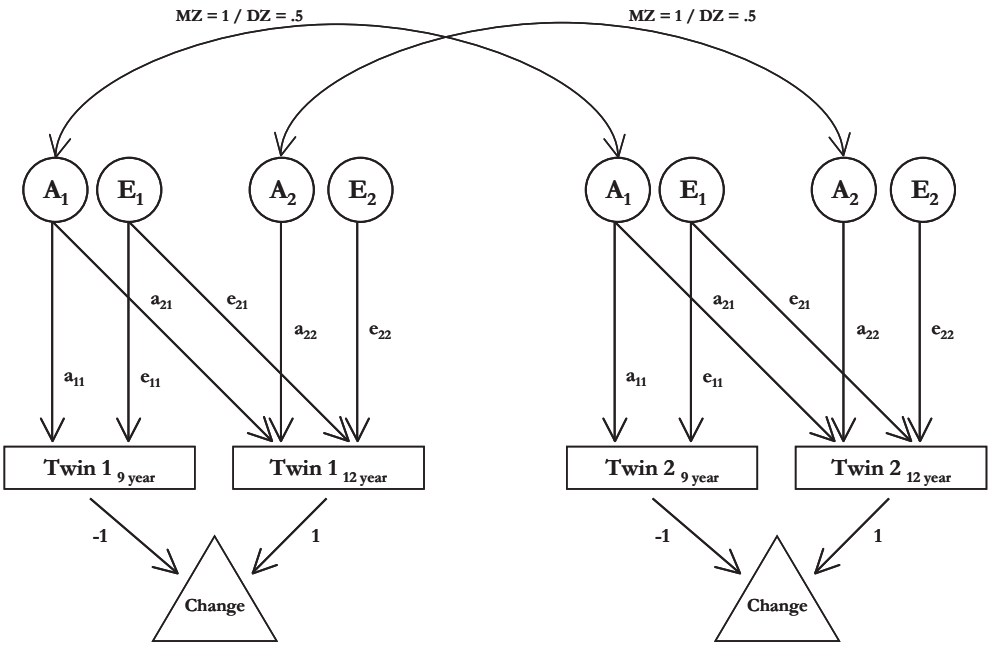

Figure 7.1 Longitudinal path diagram representing the latent (unobserved) factors A (additive genetic factor) and E (unique environment) influencing the phenotype at age 9 and 12 in both members of a twin pair. Squares represent the observed brain measurements in twin 1 and twin 2. The triangle represents the developmental change. A_1 and E_1 influence the phenotype at age 9 and 12 years; A_2 and E_2 influence the phenotype at age 12, but not at age 9 years. Double headed arrows represent correlations between the genotypes of twins (1.0 for MZ and 0.5 for DZ pairs). The influence of the first set of latent factors on the phenotype at age 9 is represented by factor loadings (one headed arrows) a_{11}, e_{11}, and at age 12 by a_{21}, and e_{21}.

Longitudinal genetic modelling

A longitudinal structural equation model was fitted to the data to estimate the influences (path coefficients) of A, C and E at ages 9 and 12. Genetic factors at age 9 can have the same influence over time, or may become more or less important with increasing age. Because common environmental influences were non-significant for all brain measures, we limit the description of the model to an AE model (see Figure 7.1).

The path coefficient a_{11} quantifies the effect of genetic influence at age 9, a_{21} quantifies the effect of genetic covariance between age 9 and 12, and a_{22} quantifies the effect of specific genes at age 12. In a similar way e_{11}, e_{21}, and e_{22} are defined. The total variance at age 9 equals $(a_{11})^2 + (e_{11})^2$. The total variance at age 12 equals $(a_{21})^2 + (a_{22})^2 + (e_{21})^2 + (e_{22})^2$. Heritability is the proportion of the total variance that can be attributed to genetic variance. Hence, heritability at age 9 is $a_{11}^2 / (a_{11}^2 + e_{11}^2)$, and heritability at age 12 is $(a_{21}^2 + a_{22}^2)/ (a_{21}^2 + a_{22}^2 + e_{21}^2 + e_{22}^2)$.

The longitudinal model (see Figure 1) also specifies a change score to gain insight into the contribution of genetic variance on individual differences in developmental change. This change score describes individual differences in developmental change from age 9 to 12. The total variance on the change score is calculated by: Variance $_{(age\ 12 - age\ 9)}$ = Variance $_{(age\ 9)}$ + Variance $_{(age\ 12)}$ – 2Covariance $_{(age\ 12,\ age\ 9)}$. Following path tracing rules in the longitudinal path diagram, the contribution of genetic variance on developmental change is calculated as $a_{11}^2 + a_{21}^2 + a_{22}^2 – 2(a_{11} * a_{21})$. Simplified this gives: $(a_{21} – a_{11})^2 + a_{22}^2$, where the first part represents the gradual change in effect size of between paths a_{11} and a_{21} of the genetic factor that is present at ages 9 and 12, and the second part represents the contribution of specific genetic influence at age 12 on total variance of cortical change.

Thus, we can observe regions where at age 12 new genetic factors are influencing cortical thickness. In this paper we refer to genetic influences that are expressed at age 12 but not at age 9 as genetic innovations (i.e., $a_{22} > 0$). Genetic factors that are expressed at both ages are referred to as stable genetic influences. These stable genetic factors can become more important (amplification) or less important between ages 9 and 12 years, as indicated by an increase or decrease in path coefficients a_{11} and a_{21}. The relative importance of stable genetic influences is referred to as the heritability due to A1. Heritability is defined as the genetic variance divided by the total variance of the phenotype. Hence there can be genetic amplification (when genetic variance at age 12 due to A_1 is larger that at age 9) and at the same time decreasing heritability (when the genetic variance is divided by a larger total variance because there also is genetic innovation and / or increased non-genetic variance).

Statistical analyses

For each subject, differences in cortical thickness across time were calculated in millimeter (mm). Differences in local cortical thickness were calculated vertex-by-vertex. The differences in cortical thickness between age 9 and 12 were tested for significance by a t-test, while adjusting the degree of freedom for familial dependence. These analyses were corrected for sex, handedness and duration of the scan-interval.

Genetic analyses were carried out by structural equation modelling (SEM) as implemented in the software package Mx (Neale et al., 2006). Parameters were estimated by maximum likelihood. This was done for each vertex of cortical thickness, while data were corrected for sex, age at scanning, and handedness effects at both time points. In order to analyze all available data, including information of incomplete twin pairs at both measurements and across time points, analyses were performed on the raw data.

A selection of regions was done in advance of the longitudinal genetic modelling based on (i) the presence of genetic influences at 9 and/or at 12 years of age (assessed by univariate genetic modelling), and (ii) the presence of cortical thinning over time. For these selection criteria correction for multiple comparisons was done according the False Discovery Rate (FDR) at a level of 0.05 (Genovese et al., 2002).

To gain insight into the genetic model for cortical thinning that best described the data we first statistically tested for the presence of genetic innovation at age 12 by constraining the parameter of interest at zero (e.g., a_{22}) and compare the model-fit with the model where the parameter was freely estimated. Second, gradual differences in effect size over time of stable genetic influences acting at ages 9 and 12 was tested by constraining the paths a_{11} and a_{21} to be equal, and compare the model-fit again with the model where these parameters were freely estimated. For the regions where we found statistical evidence of the presence of genetic innovation (i.e., a_{22} was larger than zero), this test was carried out with a_{22} as a free parameter. For the regions where we did not find genetic innovation, we tested if there were differences in the effect sizes of stable genetic influences acting at ages 9 and 12 in the absence of genetic innovations. Please note that only when a_{22} is not equal to zero and/or when a_{21} is larger or smaller that a_{11} non-zero heritability estimates for differences scores will be observed.

Comparison of models was done by likelihood ratio tests. These tests compare the differences between -2*log likelihood of the full model with that of the restricted nested model against the corresponding degrees of freedom (df). This difference has a chi-squared distribution. When a variance component was fixed at zero, a one-tailed-test was used (Dominicus et al., 2006).

Multivariate genetic modelling for selected regions

To explore whether genetic factors acting on cortical change showed an overlap between regions, we performed additional multivariate analyses on the change scores. Multivariate genetic modelling of change scores was based on cross-twin / cross-trait correlations instead of cross-twin / cross-time correlations as described in the section on study design above. Vertices were selected within regions that showed genetic influences on cortical thinning (see above). The genetic correlation (r_g) describes the extent of overlap between genetic factors acting on cortical changes between different regions.

Results

Univariate genetic modelling

Genetic influences contributed significantly to mean cortical thickness. The heritability of mean cortical thickness was 65% ($p < 0.01$) at age 9, and 82% ($p < 0.01$) at age 12. Estimates of heritability for local cortical thickness at both age 9 and 12 mapped throughout the cortex can be found in Figures 7.2 and 7.3.

At age 9, several areas of the cortex showed significant heritability estimates ranging up to 78% in the left and up to 73% in the right hemisphere. Areas that showed significant contributions of genetic influences at age 9 were located bilaterally in frontal, midsagittal frontal, temporal and inferior parietal areas, inferior and medial insula, cingulate, the anterior paracentral lobule, lingual gyrus, precuneus, cuneus, calcarine sulcus, lateral occipital areas, and left parahippocampal gyrus (FDR corrected, $p < 0.05$; see Figure 7.2).

At age 12, heritability estimates were up to 88% and 91% in left and right hemispheres, respectively. Significant heritability estimates were located bilaterally in the precuneus, left inferior, and midsagittal superior frontal areas, left anterior paracentral lobule, left inferior temporal, and left lateral occipital gyri. In the right hemisphere, significant heritability estimates were found in the superior frontal, inferior parietal, inferior temporal, and cuneus (FDR corrected, $p < 0.05$; see Figure 7.3).

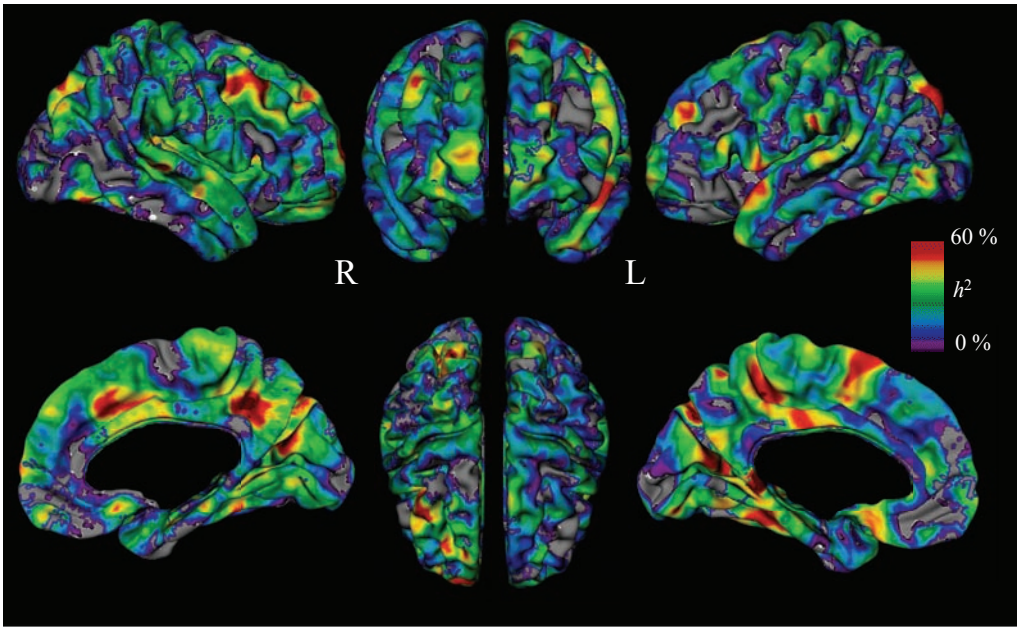

Figure 7.2 Heritability estimates (h^2) of local cortical thickness at age 9. Heritability ranged up to a maximum of 78% at age 9.

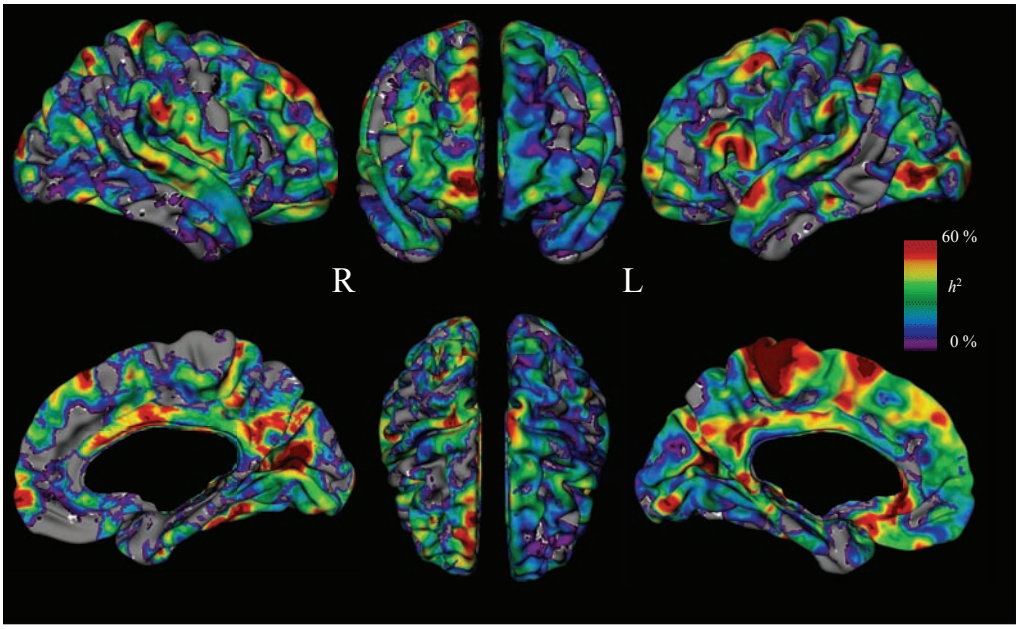

Figure 7.3 Heritability estimates (h^2) of local cortical thickness at age 12. Heritability ranged up to a maximum of 91% at age12.

Table 7.2 Cortical regions where genetic innovation at age 12 was found.

Cortical area	MNI coordinates [X, Y, Z]	Baseline h^2	Baseline Var A / Var E ($*10^{-2}$ mm^2)	Follow-up h^2	Follow-up Var A / Var E ($*10^{-2}$ mm^2)	Cortical thickness change Mean thinning (mm)	h^2	Var A / Var E ($*10^{-2}$ mm^2)	χ^2	p-value
Right medial prefrontal *	32, 58, -7	44%	2.96 /3.82	42%	1.92 / 2.64	0.10	55%	2.72 / 2.23	8.22	.002
Right superior prefrontal	14, 69, -1	45%	6.57 / 7.95	53%	6.98 / 6.21	0.19	50%	6.61 / 6.61	9.10	.001
Right inferior prefrontal *	26, 55, -15	45%	6.05 / 4.49	48%	5.58 / 6.07	0.06	55%	6.35 / 5.20	8.83	.001
Right medial frontal *	48, 34, 26	37%	1.62 / 2.81	39%	1.56 / 2.45	0.06	47%	1.71 / 1.93	5.40	.020
Right superior frontal *	4, 49, 41	35%	2.96 / 5.41	47%	2.15 / 2.43	0.03	78%	3.35 / 0.94	6.42	< .001
Right subcallosal *	4, 19, -16	44%	3.63 / 4.63	40%	2.35 / 3.52	0.19	42%	2.65 / 3.66	5.37	.010
Right cingulate	3, 24, 22	37%	4.23 / 7.20	55%	6.11 / 4.94	0.05	68%	6.22 / 2.93	6.56	.005
Right cuneus	3, -74, 22	48%	2.01 / 2.21	48%	2.08 / 2.28	0.10	51%	0.88 / 0.85	7.59	.003
Right parieto-occipital *	19, -77, 42	45%	1.74 / 2.11	42%	1.52 / 2.06	0.06	56%	1.55 / 1.22	6.44	.006
Left inferior prefrontal	-19, 68, 0	38%	4.54 / 7.47	45%	5.43 / 6.60	0.18	49%	4.72 / 4.91	8.32	.002
Left superior temporal	-63, -22, 10	44 %	2.18 / 2.80	44%	1.85 / 2.35	0.08	48%	1.24 / 1.34	6.58	.005
Left inferior parietal	-19, -88, 40	50%	2.35 / 2.33	51%	2.31 / 2.18	0.10	51%	2.17/ 2.08	8.24	.002
Left lateral occipital	-26, -91, 7	34%	2.87 / 5.48	44%	4.39 / 5.69	0.15	46%	5.42 / 6.36	7.15	.004
Left parietto-occipital	-15, -74, 36	60%	1.50 / 0.99	54%	1.36 / 1.14	0.06	58%	0.90 / 0.65	7.44	.003

Vertices are selected based on the highest value in the significant clusters ($\chi^2 > 2.71$, one-tailed test, $p < 0.05$), when path coefficient describing specific genetic variance at age 12 were fixed at zero. The heritability (h^2 %) and the unstandardized genetic and environmental variance (Var A / Var E) are given at age 9, age 12 and on cortical thinning; * For these regions, additional significant gradual differences in effect size over time of the common genetic factor across time was found ($\chi^2 > 3.84$, one-tailed test, $p < 0.05$) by constraining the paths a_{11} and a_{21} to be equal, while a_{22} was freely estimated.

Table 7.3 Cortical region with significant increase (A) or decrease (B) of stable genetic influences.

		Baseline		Follow-up		Cortical thickness change				
Cortical area	**MNI**	h^2	**Var A / Var E**	h^2	**Var A / Var E**	**Mean**	h^2	**Var A / Var E**	χ^2	**p-value**
A. Increased influence of stable genetic factor	**coordinates [X, Y, Z]**		**(* 10^{-2} mm^2)**		**(* 10^{-2} mm^2)**	**thinning (mm)**		**(* 10^{-2} mm^2)**		
Right central	11, -46, 71	13%	0.96 / 6.24	65%	4.26 / 2.32	0.08	19%	1.17 / 4.99	7.90	.005
Right calcarine	15, -74, 12	49%	2.26 / 2.33	76%	4.36 / 1.39	0.25	10%	0.34 / 3.06	7.26	.007
Right inf isthmus	22, -39 , -12	34%	1.03 / 2.02	60%	1.91 / 1.29	0.07	10%	0.14 / 1.26	10.61	.001
Left anterior paracentral	-2, -33, 67	30%	2.08 / 4.79	82%	7.71 / 1.66	0.09	34%	1.78 / 3.14	19.75	< .001
Left inferior frontal	-41, 30, 18	10%	0.30 / 2.79	51%	1.29 / 1.24	0.06	15%	0.34 / 1.93	5.65	.017
Left rostral cingulate	-4, 30, -7	20%	1.50 / 5.98	66%	4.36 / 2.23	0.12	31%	1.64 / 3.65	6.48	.011
Left inferior parietal	-55, -51, 45	9%	0.56 / 5.77	59%	3.51 / 2.47	0.05	35%	1.84 / 3.42	8.18	.004
Left central	-12, -44, 71	31%	1.38 / 3.07	72%	3.96 / 1.57	0.08	43%	1.32 / 1.75	13.36	< .001
B. Decreased influence of stable genetic factor										
Right superior prefrontal	12, 66, 11	55%	7.44 / 6.03	9%	0.90 / 8.95	0.12	42%	5.23 / 7.22	16.24	< .001
Right medial prefrontal	29, 61, -9	53%	4.18 / 3.66	24%	1.64 / 5.14	0.11	49%	3.91 / 4.07	12.50	< .001
Right medial frontal	39, 26, 41	37%	2.06 / 3.54	27%	1.01 / 2.70	0.04	55%	1.93 / 1.58	9.41	.002
Right inferior parietal	29, -70, 37	52%	1.92 / 1.80	5%	0.11 / 2.41	0.08	49%	1.10 / 1.14	13.70	< .001
Left cingulate sulcus	-14, -42, 48	59%	1.93 / 1.35	22%	0.68 / 2.41	0.06	19%	0.32 / 1.36	10.39	.001
Left anterior cingulate	-13, 41, 16	38%	2.13 / 3.47	9%	0.42 /4.04	0.08	23%	0.66 / 2.21	6.51	.011
Left frontal pole	-18, 64, 9	37%	4.62 / 7.85	26%	2.65 / 7.50	0.12	37%	3.69 / 6.28	5.99	.014
Left inferior temporal	-58, -56, -15	53%	4.11 / 3.58	3%	0.22 / 6.46	0.06	37%	2.43 / 4.14	15.27	< .001
Left inferior parietal	-25, -84, 37	60%	2.37 / 1.58	4%	0.14 /3.50	0.11	14%	0.32 / 1.97	15.56	< .001

Vertices are selected based on the highest value in the significant clusters ($\chi^2 > 3.84$, two-tailed test, $p < 0.05$), when path coefficient describing variance at age 9 and variance of that factor at age 12 are constrained to be equal. The heritability (h^2 %) and the unstandardized genetic and environmental variance (Var A / Var E) are given at age 9, age 12 and on cortical thinning.

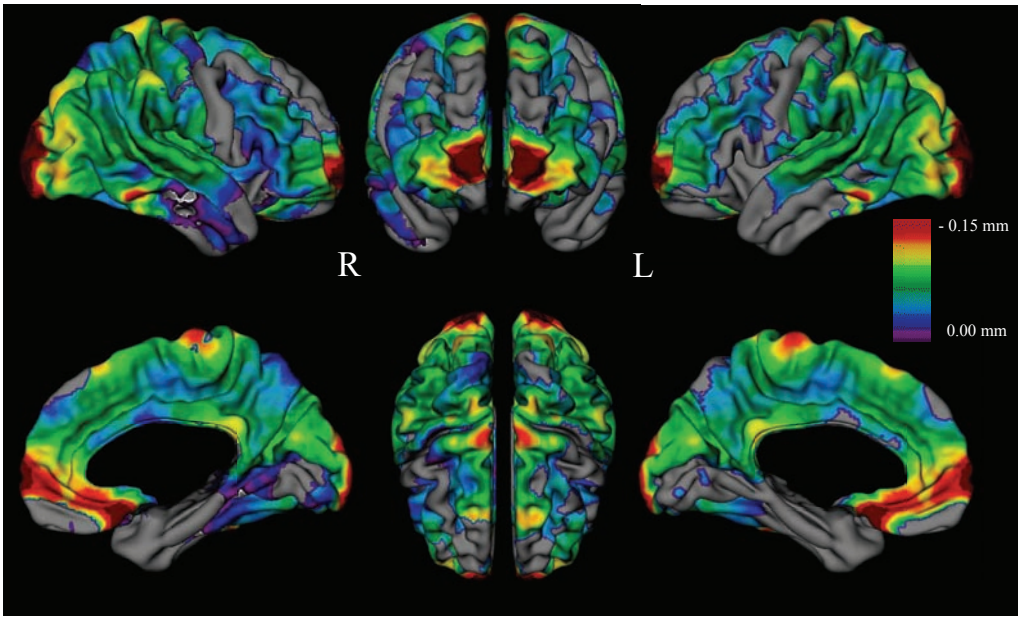

Figure 7.4 The average thinning of local cortical thickness between 9 and 12 years old. Decrease of each vertex is given in mm, corrected for handedness, sex, and interval duration. Depicted are regions where cortical thinning was significantly different from zero for the left (FDR corrected; p< .033) and right hemispheres (FDR corrected; p < .039). Maximum thinning was 0.24 mm in the occipital cortex.

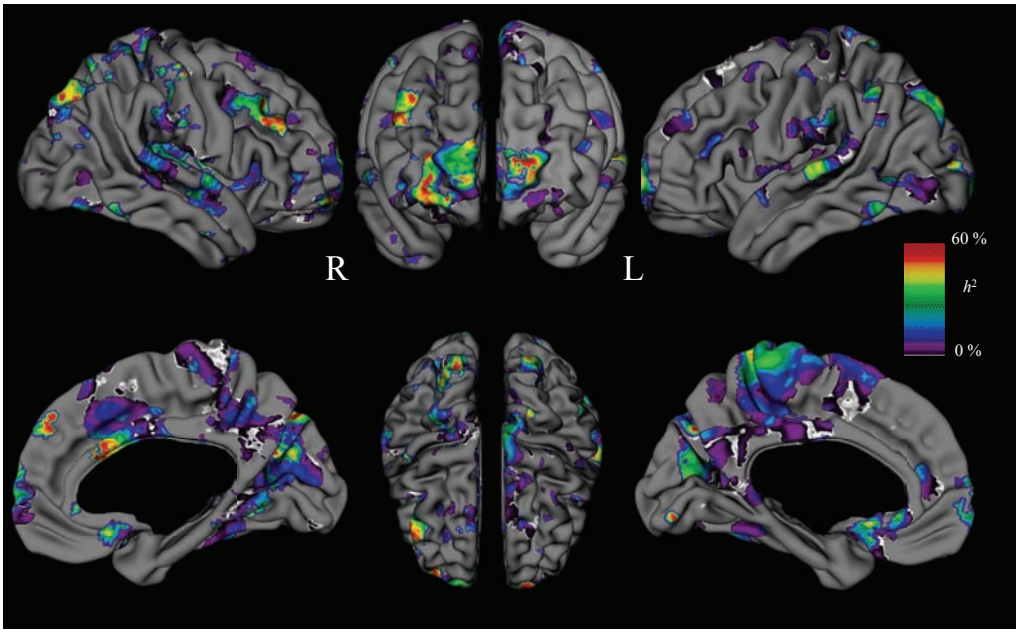

Figure 7.5 Heritability estimates (h^2) of local cortical thinning between age 9 and 12. Areas are shown only when there was significant thinning, and significant genetic influences at age 9 or at age 12 (FDR corrected) for left (L) and right (R) hemispheres.

Because heritability is defined as a proportion of the total variance, changes in the unstandardized values of the genetic and environmental components and the total variances were evaluated between age 9 and 12. The total variance showed an overall decrease across the cortex between the two measurement points. This was mostly due to decreased unique environmental influences. It should be noted that regions of increased and decreased heritability coincide with an actual increase or decrease of unstandardized genetic variance.

Developmental changes

A significant decrease in mean cortical thickness of 0.05 mm ($p < 0.01$) was observed between ages 9 and 12. No significant heritability of mean cortical thinning was found. Differences in local cortical thickness between 9 and 12 years were also calculated for each vertex separately and significant cortical thinning was observed throughout the cortex (FDR corrected, $p < 0.05$). The amount of thinning was widely distributed over the different regions, reaching a maximum of 0.24 mm thinning, and was most pronounced in frontal pole, parietal and lateral occipital cortices (Figure 7.4). There were no areas with significant cortical thickening.

Heritability of cortical thinning

For cortical areas where significant cortical thinning and significant genetic influences on cortical thickness at age 9 and/or 12 were found (all based on FDR corrected data, leaving 7841 vertices in the left hemisphere, and 10173 vertices in the right hemisphere, see Figure 7.6 for areas that were included), genetic influences on individual differences in cortical thinning were explored in the longitudinal path model as depicted in Figure 7.1. Heritability estimates of cortical thinning locally were up to 79% for the right, and 61% for the left hemisphere, and were predominantly found in superior and middle frontal areas, superior temporal areas, cingulate, sensorimotor cortices, primary visual and lateral occipital cortices (Figure 7.5). The next step was to explore what caused the heritability estimates found on cortical thinning.

First, the presence of significant genetic innovation at age 12 was evaluated. Areas with genetic innovation were located in bilateral superior frontal areas, parieto-occipital sulcus, right medial frontal areas, and anterior cingulate, left superior temporal, and lateral occipital areas ($\chi^2 > 2.71$, one-tailed test, $p < 0.05$; see Figure 7.6). Of the regions where genetic innovation was found, the vertices with the highest χ^2 differences were selected and model fitting results are given in more detail in Table 2.

Second, in the regions where variance in cortical thickness between age 9 and 12 could be explained by stable genetic influences (i.e., no genetic innovation at age 12), we tested whether stable genetic influences across time had an increasing or decreasing influence between age 9 and 12. Significant increasing effect sizes of stable genetic influences were found at the right central sulcus, calcarine sulcus, inferior isthmus, and left anterior paracentral lobule, rostral cingulate, inferior parietal lobule, and inferior frontal gyrus ($\chi^2 > 3.84$, two-tailed test, $p < 0.05$; Figure 7.6, see, Table 7.3A). Areas where stable genetic influences were significantly decreasing in magnitude were located in bilaterally inferior parietal, right inferior parietal, medial and superior pre-frontal, left anterior cingulate sulcus, frontal, and inferior temporal areas (Figure 7.6, Table **7.**3B).

Multivariate genetic modelling

To explore the overlap of genetic factors acting on cortical thinning between different regions, single vertices were selected based on the findings described above (see also Figure 7.6). Phenotypic and genetic correlations between the selected regions on cortical thickness change scores are given in Table 7.4. For regions with genetic innovation, cortical changes were genetically correlated between frontal regions bilaterally ($r_g = 0.83$). For regions where stable genetic influences of increasing magnitude were observed, the genetic factor acting on cortical change in left inferior parietal showed a complete overlap with the genetic factor acting on cortical change in left inferior frontal region ($r_g = 1.00$). These genetic factors were independent from the genetic factor acting on cortical changes in left anterior paracentral lobule. In selected regions with a stable genetic influence that decreased in importance no genetic overlap was found between the selected regions.

Two vertices located anatomically close to each other in the right frontal region, were chosen to study in more detail (see Figure 7.7). For one of these cortical regions, stable genetic influences were found to act on cortical thickness at both points in time ($r_g = 1.00$), and this genetic factor decreased in importance over time (Figure 7.7; vertex 1). For a region close to vertex 1, genetic innovation was found (Figure 7.7; vertex 2). The common genetic factor at vertex 1 showed a significant overlap with the genetic factor at age 9 of vertex 2 ($r_g = 0.80$), and no significant overlap with the genetic factor at age 12 of vertex 2.

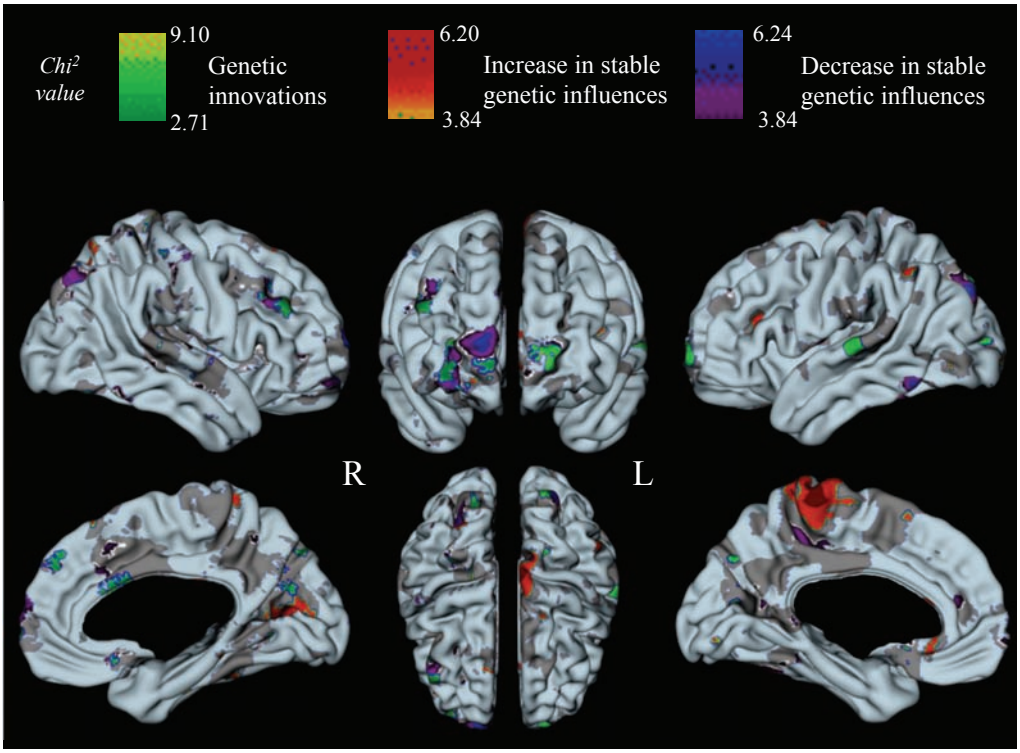

Figure 7.6 Cortical regions where changes in genetic influences at different points in time were found. White areas represent vertices that were not included in this analysis; either because there was no significant cortical thinning, or because there were no significant contributions of genetic factors at either age 9 or 12. Chi-square values are given for the different statistical tests for left (L) and right (R) hemisphere respectively. Regions of genetic innovations at age 12 (green) and gradual differences in effect sizes of stable genetic influences with age (blue and red).

Table 7.4 Phenotypic and genetic correlations between regions of interest on cortical thickness changes.

A. Genetic innovation	**MNI coordinates [X, Y, Z]**	L Superior Temporal	R Frontal	L Frontal
L Superior Temporal	-63, -22, 10	-	*0.00*	*0.22**
R Frontal	14, 69, -1	-0.01	-	*0.50**
L Frontal	-19, 68, 0	0.55	0.83*	-

B. Increase in stable genetic influences		L Inferior Frontal	L Inferior Parietal	L Anterior Paracentral lobule
L Inferior Frontal	-41, 30, 18	-	*0.30**	*0.15*
L Inferior Parietal	-55, -51, 45	1.00 *	-	*0.21**
L Anterior Paracentral lobule	-2, -33, 67	-0.23	-0.18	-

C. Decrease in stable genetic influences		R Frontal	R Inferior Parietal	L Inferior Parietal
R frontal	12, 66, 11	-	*0.09*	*0.20**
R Inferior Parietal	29, -70, 37	0.12	-	*0.28**
L Inferior Parietal	-25, -84, 37	0.34	0.18	-

L = left side; R = right side; * Significant genetic correlations ($p > 0.05$). Single vertices were selected within areas of genetic innovation at age 12 (A), increasing (B), and decreasing (C) stable genetic influences across time. Off diagonal above (italic) are the phenotypic correlations between the different regions and off diagonal below the genetic correlations are given.

Discussion

In this longitudinal study we scanned a large group of twins at ages 9 (N = 190) and 12 (N = 125). We found considerable thinning of the cerebral cortex in this three year interval of 0.05 mm on average (1.5%). Cortical thinning was most pronounced in the frontal pole and orbito-frontal, sensory-motor and visual cortices during this period. Finally, we demonstrated that this process is highly heritable (up to 79%). This was locally driven by independent genetic factors, both between brain regions, and across time. Variation in thinning of the left inferior frontal (Broca's area) and left parietal (Wernicke's area) cortices was driven by the same genetic factor. This factor was independent from the genetic factor influencing left anterior paracentral (sensory motor) cortical thinning. Cortical thinning in the left and right frontal poles is driven by two genetic factors: one factor of decreasing influence over time, and another independent genetic factor at age 12 in left and right frontal poles. Thus, developmental thinning of the cerebral cortex in children and adolescents (Gogtay et al., 2004) is heritable in children developing into adolescents. Different genetic factors are responsible for variation in cortical thickness at ages 9 and 12, and different genetic factors are exerting influences across time and between various brain areas.

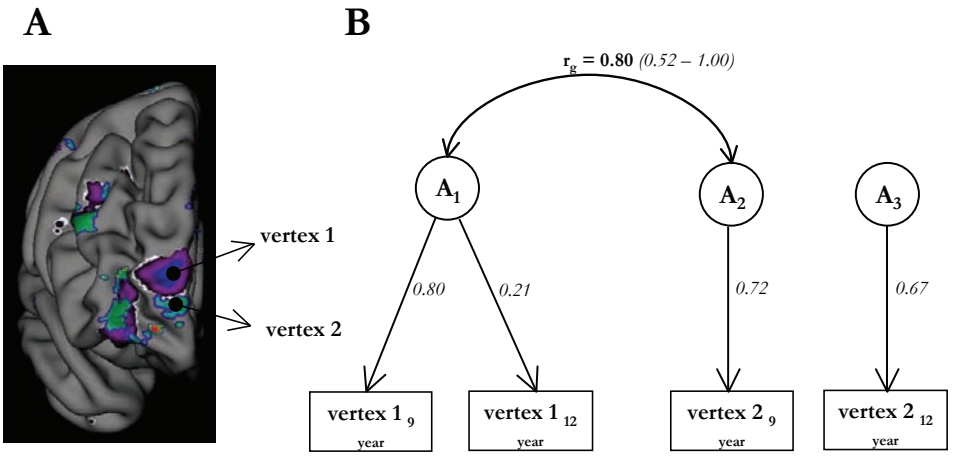

Figure 7.7 Two vertices were chosen, that were located anatomically close to each other in the right frontal region (A). Diagram illustrating the outcome of mulitvariate analyses between these two regions of interest (B). Squares represent the observed cortical thickness in vertex 1 and vertex 2 at both ages 9 and 12. The double headed arrow represents the genetic correlation between the genetic factors modelled. For vertex 1, stable genetic influences were found to act on cortical thikness at both points in time (r_g = 1.00), and this genetic factor decreased in importance over time. For vertex 2, genetic innovation at age 12 was found. The common genetic factors at vertex 1 showed a significant overlap with the genetic factor at age 9 of vertex 2 (r_g = 0.80), and no significant overlap with the genetic factor at age 12 of vertex 2.

To the best of our knowledge this is the first longitudinal study on genetic and environmental contributions to cortical gray matter changes during childhood. Because of the genetically informative longitudinal design, it was possible to explore how genetic mechanisms exert their effects on individual differences in cortical development. This study shows not only that cortical thinning is heritable in adolescence, as previously reported in adulthood (Brans et al., 2010), but that between ages 9 and 12 cortical thinning is driven by different genetic factors exerting their influence on particular areas of the cerebral cortex. That we find overall thinning between 9 and 12 years in this sample of children with an average IQ of 101 (van Soelen et al., 2011) is consistent with earlier findings in a sample of children between 4 and 20 years where cortical thinning was also found to have started before age 12 (Shaw et al., 2006). It must be noted that the exact mechanism behind cortical thinning in adolescence is not known. Cortical thinning during puberty has been associated with the loss of unneeded connections (synaptic pruning; Huttenlocher and Dabholkar, 1997) but pruning cannot fully account for the observed thinning (Paus, 2005). Decrease in gray matter and the increase in white matter is also thought to be a reflection of progressive age-related axonal myelination (Benes et al., 1994; Yakovlev and Lecours, 1967), which is assumed to occur at the gray-white matter boundary (white matter encroachment; Gogtay and Thompson, 2010).

One other interesting finding is the suggestive evidence for waning influence of one genetic factor related to cortical thickness in the left and right frontal pole and the emerging activation of another genetic factor in the same regions (from 9 to 12 years). The frontal pole has been specifically

associated with the evolution of the human species since it is one of the cortical areas of the frontal lobe that is enlarged in humans as compared to apes (Semendeferi et al., 2001). Furthermore, the frontal pole, or Brodmann Area 10, has also been associated with many cognitive functions, principally multitasking and dealing with novel situations (Dumontheil et al., 2008). Mentalizing, Theory of Mind, and prospective memory are important cognitive functions in which the frontal pole is involved and have been reported to show changes in cognitive strategies around this age period (Ward et al., 2005).

Bilateral thinning was considerable in the region on the top of the mesial wall of the paracentral lobule in this 3-year period. An amplification of this stable genetic factor was found on the thickness of the primary sensory-motor cortex. Intriguingly, this brain area has recently been found to be activated during genital stimulation, both in females (Michels et al., 2010) and in males (Kell et al., 2005), despite earlier suggestions that representation of primary sensory-motor for the sexual organs were located below the sensory-motor area of the toe (Penfield and Rasmussen, 1950). Thus, while we can only speculate if increasing genetic influence on cortical thinning of the top of the mesial wall of the paracentral lobule is indeed targeting development of sensory-motor representation of the penis and clitoris in the brains in boys and girls, the period between 9 and 12 years does represent the start of sexual maturation (Marshall and Tanner, 1969; 1970). To which extent genes implicated in development of this primary sensory-motor cortical area overlap with those implicated in the development of sexual hormones is of interest for future study.

The same genetic factor operates in language areas (e.g., left inferior frontal (superior of) Broca's and left parietal (superior of) Wernicke's area). In these areas amplification of the same genetic factors across ages 9 and 12 was observed. Between these two regions, the genetic factors acting on cortical thinning were completely overlapping and this might suggest that the genetic influences acting on cortical development in these areas are implicated in language.

We can only speculate which specific genes are represented by the genetic factors estimated in the present study. There are examples of genes that are expressed throughout life and show a change in their expression pattern with maturation. For example, Brain-Derived Neurotrophic Factor (BDNF), is highly expressed in the cerebral cortex, and has an important role during brain development and in synaptic plasticity (Cohen-Cory et al., 2010). The expression pattern of BDNF was found to be unique for different anatomical regions, and coincides with maturation timing of different anatomical regions of the cortex (Webster et al., 2002; Webster et al., 2006; Wong et al., 2009). Similar changes in expression levels in the prefrontal cortex were found for the expression of dopamine receptors (DAR1), $GABA_A$ receptor alpha-subunits, and Apolopoprotein-D (Duncan et al., 2010; Kim et al., 2009; Weickert et al., 2007). Thus, expression levels of genes can change during life and this could be one interpretation of the gradual changes or the upcoming different genetic factors between age 9 and 12 observed in the present study.

We did not find significant influences of common environmental factors on cortical thickness. This is consistent with other neuroimaging studies in twins, indicates that common environmental effects are not significant in adults (Peper et al., 2007), and in children (Lenroot et al., 2009; Peper et al., 2009; Yoon et al., 2010) for both volumetric and cortical thickness measures. The narrow age range of our cohort makes it possible to assess genetic influences without age-related confounders, providing accurate heritability estimates at 9 and 12 years of age. The relatively high heritabilities for mean and local cortical thickness reported in the present study are consistent with one other study in children (Yoon et al., 2010), as well as with the impression that heritability increases with age (Lenroot et al., 2009). When interpreting the findings of this study we should keep in mind however that the heritability estimates are based on local vertices and therefore dependent on local accuracy of the measurement. Local inaccuracies leading to noise in the data may have resulted in local overestimations and, more likely, underestimations of the influence of genes.

Conclusions

This study shows the extent to which genetic factors can influence cortical changes during early puberty between 9 and 12 years of age. Importantly, besides gradual differences in contribution of stable genetic influences acting on cortical thickness, we find evidence for independent genetic influences acting on cortical thickness at different points in time. These findings can be interpreted as changes in gene expression patterns in various brain regions during cortical development in childhood. The complex interplay between genes, hormones and environment is important to fully understand how individual differences in healthy brain development arise. Understanding how genetic and environmental influences can act on normal brain development, can contribute to the understanding of the complex biological processes underlying healthy, but also disrupted brain development.

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8

Associations between intelligence and cortical thickness emerge at the start of puberty

This chapter is based on:
I.L.C. van Soelen*, R.M. Brouwer*, H.G. Schnack, R.S. Kahn, H.E. Hulshoff Pol*, D.I. Boomsma* (* Equal (first/ last) authorship). In preparation.

Abstract

General cognitive abilities are related to brain structure and to structural brain changes during adolescence and adulthood. Findings from previous studies suggest that there may be different associations between cortical thickness and intelligence at different ages. In the present study, the association between brain structure and IQ (full scale, verbal and nonverbal IQ) was explored in a longitudinal pediatric twin sample at two ages, namely in childhood (age 9) and early adolescence (age 12). In addition, it was studied to what extent these associations are caused by shared genetic factors acting on both cortical thickness and intelligence. Intelligence was assessed in 224 twins at age 9, and 177 at age 12, of which 190/125 twins had magnetic resonance imaging (MRI) scans available. Cortical thickness per region of interest (78 ROIs) was measured at both ages. At age 9, cortical thickness was not correlated with intelligence scores. At age 12 a correlation emerged with intelligence, with higher IQ scores associated with a thinner cortex. This effect was mainly driven by verbal IQ, with the strongest correlations located in the left frontal cortex. The correlations between IQ and cortical thickness were driven by shared genetic factors. In conclusion, it seems that brain areas contributing to (verbal) intellectual performance are specializing with the onset of puberty, driven by genes influencing both IQ and cortical thickness.

Introduction

The transition from childhood into adolescence is characterized by cognitive maturation, accompanied by considerable structural brain changes (e.g., Casey et al., 2005; Giedd et al., 1999). Individual differences in general cognitive functioning are increasingly influenced by genetic factors from childhood to adolescence. The influence of common environment is evident in young childhood, but decreases and eventually disappears during adolescence (Bartels et al., 2002; Haworth et al., 2010). The contribution of common environmental factors to intelligence in childhood acts mainly on the verbal counterpart of intelligence (Hoekstra et al., 2007; van Soelen et al., 2011c). Apart from the cognitive maturation, considerable changes in brain structure take place around puberty (Giedd et al., 1999; Sowell et al., 2002). Although white matter of the brain is still increasing in volume, gray matter volume starts to decrease around puberty (Giedd et al., 1999). Decrease in gray matter volume is most often associated by overall thinning of the cortex (Sowell et al., 2002; Gogtay et al., 2004).

Not only is brain size (e.g., McDaniel, 2005) associated with intelligence, cortical thickness is also associated with general cognitive functioning in children (Karama et al., 2011), and in adults (Narr et al., 2007; Brans et al., 2010). In adulthood, a higher intelligence was accompanied by a thicker cortex (Narr et al., 2007; Brans et al., 2010). A similar result has been found in a cross-sectional sample consisting of children between young childhood and late adolescence (Karama et al., 2011). A pioneering study has shown that intelligence is related to the trajectory of cortical development during childhood and adolescence, rather than cortical thickness itself (Shaw et al., 2006). In this study a developmental shift from a predominantly negative correlation between intelligence and cortical thickness in early childhood changed into a positive correlation in late childhood and adolescence. These findings suggest that there may be different associations between cortical thickness and intelligence at different ages.

We studied the association between intelligence and cortical structures (i.e., cortical thickness) in a population based longitudinal twin sample at two specific ages in the end of childhood (9 years old), and in early adolescence (12 years old). Full scale, verbal and nonverbal intelligence (Hoekstra et al., 2007; Posthuma et al., 2001; van Soelen et al., 2011c) and cortical thickness (Yoon et al., 2010; van Soelen et al., 2011b; Lenroot et al., 2009; Kremen et al., 2010; Panizzon et al., 2009; Brans et al., 2010) are heritable traits. Given the associations between brain structure and intelligence in children, we ask the question to what extent the same genes influence these two traits. Other measures of cortical morphology (i.e., cortical surface and gray matter volume) are explored and compared to the findings with cortical thickness and intelligence, to investigate how specific the association with intelligence is for the thickness of the cortex.

Materials and methods

Setting

Intelligence was assessed in a large sample of twins at ages 9 and 12. At both measurements children underwent structural MRI scanning, and cortical thickness was measured. Previous publication on this data at baseline showed that brain volumes were positively associated with intelligence at the age of 9 (van Leeuwen et al., 2009). Data on the genetic architecture of intelligence in these twins and their siblings at both baseline and follow-up can be found in van Soelen, et al. (2011c). In this sample of children of average intelligence, widespread cortical thinning is already present between ages 9 and 12, and variation in thinning is determined by genes (van Soelen et al., 2011b).

Subjects

All twins were recruited from the Netherlands Twin Register (NTR; Boomsma et al., 2006). In total 112 twin pairs were included at baseline with a mean (s.d.) age of 9.1 (0.1) years. Exclusion criteria consisted of chronic use of medication, any known major medical or psychiatric history, or participation in special education. At follow-up, 89 twin pairs came back at a mean age of 12.1 (0.3) years.

Intelligence was assessed in 224 twins at baseline and 177 at follow-up, of which 190/125 twins had magnetic resonance imaging (MRI) scans available at baseline and follow-up, respectively. Drop-out for the MRI scans usually resulted from having metal material in the head, i.e. dental braces. At baseline/follow-up the sample included 96/80 monozygotic (MZ), 88/68 dizygotic same-sex (DZ), and 40/29 dizygotic opposite sex (DOS) twins (all complete twin pairs, with the exception of one DOS twin with missing IQ data at follow-up), of which 82/56 MZ twins (38/23 complete twin pairs), 75/45 same-sex DZ twins (32/18 complete twin pairs), and 33/24 DOS twins (14/10 complete twin pairs) completed the scan protocol successfully.

Average time between intelligence testing and MRI scan was 50 days at baseline, ranging from 4 up to 128 days. At follow-up, the intelligence test and the MRI scan were conducted at the same day. Written informed consents were obtained from all subjects and their parents and the study was approved by the Dutch Central Committee on Research involving Human Subjects (CCMO). Parents were financially compensated for travel expenses and the children received a small gift each.

Intelligence measures

At baseline the full version of the Wechsler Intelligence Scale for children –Third version (WISC-III; Wechsler et al., 2002) was used, including six verbal and six nonverbal subtests. At follow-up a shortened version of the WISC III was administered (as previously described by van Soelen et al., 2011c), including four verbal and two nonverbal subtests. Test scores were corrected for the different number of subtests. Raw scores were standardized according the age of the child at moment of testing, based on a population sample of same-aged subjects in the Netherlands, resulting in full scale, verbal and nonverbal intelligence quotients (IQ).

Image acquisition

Scanning and the subsequent image processing took place at the University Medical Center Utrecht, The Netherlands. To limit possible effects of scanner instability over time, the same scanner parameters as well as image processing procedures were applied at both ages. All structural magnetic resonance imaging (MRI) was performed on a 1.5-T Philips Achieva scanner at both ages. All children underwent a practise session in a dummy scanner in advance to become familiar with the scanning procedure (Durston et al., 2009). At both measurements image-sequences of the whole head were acquired, including a short scout scan for immediate verification of optimal head positioning, and a clinical scan that was used for neuro-diagnostic evaluation. Three-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head (256 × 256 matrix, TE = 4.6 ms, TR = 30 ms, flip angle = 30°, 160-180 contiguous slices; 1 × 1 × 1.2 mm^3 voxels, Field-of-View = 256 mm / 70%) was acquired for volumetric analysis and assessment of cortical thickness.

Image processing

Scans were put into Talairach frame (no scaling), and corrected for inhomogeneities in the magnetic field (Sled et al., 1998). Quantitative assessment of intracranial volume (IC) was performed as previously described for baseline (Peper et al., 2008), and follow-up (van Soelen et al., 2011a). Cerebral spinal fluid, gray and white matter were segmented using a partial volume segmentation method incorporating a non-uniform partial volume distribution (Brouwer et al., 2010). Total gray matter volume was subsequently divided into cortical and subcortical gray matter volume. Cortical thickness and surface area were assessed using a custom implementation of the CLASP algorithm, designed at the McConnell Brain Imaging Centre, Montreal (Kim et al., 2005; Lerch et al., 2008), which started from the gray and white matter segments created by our own algorithm as described above. A surface consisting of 81,920 polygons and 40,962 vertices was fitted to the white matter/gray matter interface of each subject's left and right hemisphere, which was then expanded out to fit the gray matter/cerebrospinal fluid interface, thereby creating the outer cortical surface. The area of the cortex was estimated from the surface half-way between the gray/white interface and the outer surface of the cortex. The surfaces of the subjects were registered to an average surface (International Consortium for Brain Mapping; Lyttelton et al., 2007), allowing comparison of cortical thickness locally between subjects. The automated anatomical labelling (AAL) atlas (Tzourio-Mazoyer et al., 2002), defined on this surface, was used to compute mean cortical thickness per region of interest (78 ROIs; 39 for both left and right hemispheres).

Statistical analyses

All data analyses were carried out with structural equation modelling (SEM) in the software package Mx (Neale et al., 2006). All available data were analyzed, i.e. regardless whether subjects participated once or twice in the study, or when data were incomplete within a twin pair. All imaging data were corrected for sex, age and handedness. First, phenotypic correlations were estimated between IQ and cortical thickness at each ROI at both ages 9 and 12 separately, while correcting for familial dependencies. For the phenotypic correlations between IQ and the different ROIs, a correction for multiple comparisons was done according to the False Discovery Rate (FDR) at a level of 0.05 (Genovese et al., 2002). Second, to determine to what extent the correlations between IQ and cortical thickness were due to genetic or environmental influences, a bivariate genetic model was fitted to the data.

Bivariate genetic modelling

Twin studies are widely used to quantify to what extent genetic and environmental factors influence individual differences found in the population, by comparing the similarities within monozygotic (MZ; share (nearly) 100% of their genetic material) and dizygotic (DZ; share on average 50% of their segregating genes) twin pairs. Additive genetic influences (A) represent the influences on the phenotype of multiple alleles at different loci on the genome that act additively. Common environmental influences (C) include all environmental effects that make members of the same family resemble each other. Unique environmental influences (E) are not shared with other family members (Plomin et al., 2001). A larger correlation between members of MZ pairs compared to DZ pairs indicates genetic influences on a certain trait.

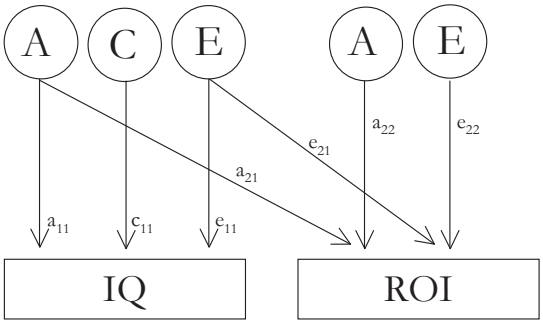

Figure 8.1 Path diagram representing the bivariate genetic model. Squares represent the measured variables (i.e., IQ and cortical thickness (ROI)), and the circles represent latent, unobserved factors. The influence of the first set of latent factors on intelligence is represented by the path coefficients (one-headed arrows) a_{11}, c_{11}, and e_{11}, and on the cortical thickness by a_{21}, c_{21}, and e_{21}. The second set of latent factors influence cortical thickness only, represented by the path coefficients a_{22}, c_{22} and e_{22}. Variance IQ $=(a_{11})^2 +(c_{11})^2 + (e_{11})^2$. Variance ROI $=(a_{21})^2 + (a_{22})^2 + (e_{21})^2 + (e_{22})^2$. The covariance between IQ and ROI $= (a_{11} * a_{21}) + (e_{11} * e_{21})$. In MZ twins the genetic factors are correlated 1.0 within twin pairs and 0.5 for DZ. In MZ, DZ and twin-siblings, the common environmental factors are correlated 1.0, and the unique environmental factors are uncorrelated.

A bivariate genetic model between two traits allows for estimation of cross-twin/cross-trait correlations, i.e., to what extent the IQ of one twin correlates with its co-twin's cortical thickness. Larger MZ cross-correlations than DZ cross-correlations indicate that there is an overlap between the genetic factors for IQ and cortical thickness. If MZ cross-correlations are not larger than DZ cross-correlations there is no such overlap in genetic factors between the two traits.

Based on previous research, it is known that there are no influences of common environment on variability of volumetric measures (van Soelen et al., 2011a) and cortical thickness (van Soelen et al., 2011b). Therefore, no common environmental influences were modeled for the brain structures. Figure 8.1 represents the bivariate genetic model, where cortical thickness as in each ROI can be inserted as the second measured variable. Based on this model, total variance of IQ equals $(a_{11})^2 +(c_{11})^2 + (e_{11})^2$, and the total variance of the brain structure, e.g., cortical gray matter volume or cortical thickness in each ROI, as illustrated in Figure 8.1, equals $(a_{21})^2 + (a_{22})^2 + (e_{21})^2 + (e_{22})^2$. The covariance between IQ and cortical thickness in the ROI is derived from multiplying the path coefficients that define the association between the two phenotypes. The total covariance is a summation of these covariances, namely $(a_{11} * a_{21}) + (e_{11} * e_{21})$. The extent to which genetic influences on IQ and cortical thickness in the ROI overlap can be calculated as the genetic correlation $r_g = (a_{11} * a_{21}) / \sqrt{(a_{11}^2 * (a_{21}^2 + a_{22}^2))}$. In a similar way the unique environmental correlation can be obtained. All correlations were tested to establish if they significantly contributed to the association between IQ and cortical thickness in the ROI by constraining a_{21}, or e_{21} at zero. Tests of significance of parameters were carried out by comparing the modelfits of the model including that parameter, and the model when the estimate was constrained at zero. The goodness of fit of different models was evaluated by comparing differences in log-likelihood. Twice the difference between log likelihoods is chi-squared distributed with degrees of freedom (df) equal to the difference in the number of parameters estimated in two models.

Results

Mean (s.d.) full scale IQ was 99.9 (13.5) at age 9, and 100.3 (14.1) at 12. Mean level of verbal and nonverbal IQ was 99.6 (14.8) and 100.1 (12.3) at age 9. At age 12, children had an average verbal and nonverbal IQ of 102.3 (12.5), and 98.36 (17.6), respectively. IQ scores were correlated between ages 9 and 12 for full scale ($r = 0.70$), verbal ($r = 0.71$), and nonverbal IQ ($r = 0.54$). Mean cortical thickness was 3.36 (0.09) mm at age 9 and 3.30 (0.09) mm at age 12. Verbal and nonverbal IQ were correlated at ages 9 ($r = 0.52$) and 12 ($r = 0.40$), and were significantly different from 1.00 at both ages.

Correlations between IQ and cortical thickness

Phenotypic correlations between full scale IQ and cortical thickness in the different ROIs are given in Figure 8.2 and 8.3. Overall, negative correlations were observed between cortical thickness and full scale IQ, and were much more pronounced at age 12, compared to age 9. Regions in which cortical thickness was correlated with IQ ($p < 0.05$) are given in Table 8.1 for age 9 and age 12. At age 12, negative phenotypic correlations between cortical thickness and IQ reached up to -0.32. After FDR correction for multiple comparisons, no ROI reached significance at age 9. A considerable number of correlations between IQ and cortical thickness survived FDR correction at age 12 (see Table 8.1).

The phenotypic correlations between cortical thickness and verbal and nonverbal IQ are presented in Figure 8.4 and Figure 8.5, respectively. Comparing verbal IQ to nonverbal IQ, phenotypic correlations between intelligence and cortical thickness seem to be driven by the verbal counterpart of IQ, rather than the nonverbal counterpart (see Tables 8.2 and 8.3). Significant negative correlations between verbal IQ and cortical thickness are found at age 12, but are absent at age 9. These correlations are more widespread in the left hemisphere (mostly in frontal areas), compared to the right hemisphere. Similar to verbal IQ, a larger number and stronger negative correlations were found at age 12 compared to age 9 for nonverbal IQ, but only one correlation at age 12 survived FDR correction (occipital middle area).

As there were small differences in the data collection between baseline and follow-up, it is of importance to exclude these possible confounders on the observed and non-existent correlations between IQ and cortical thickness. Therefore, the effects of children who did not return at the follow-up (drop-out), the difference in the amount of subtests between the two moments of testing, and the amount of time between IQ assessment and MRI scanning were explored in more detail. It is possible that at age 9, the children who did not return at follow-up, obscured the correlation emerging at age 12. In regions that showed such a correlation at age 12, we repeated the analysis at age 9, including only children that also returned at follow-up. Still, no significant correlations at age 9 were observed (p's > 0.05). Similarly, using only the IQ subtests that were included at age 12 and repeating the analyses at age 9 showed no significant correlations between intelligence and cortical thickness. Further, the differences in the time period between the cognitive assessment and moment of scanning at baseline could not explain the absence of a correlation at age 9 compared to age 12, where IQ tests and MRI acquisition was done at the same day.

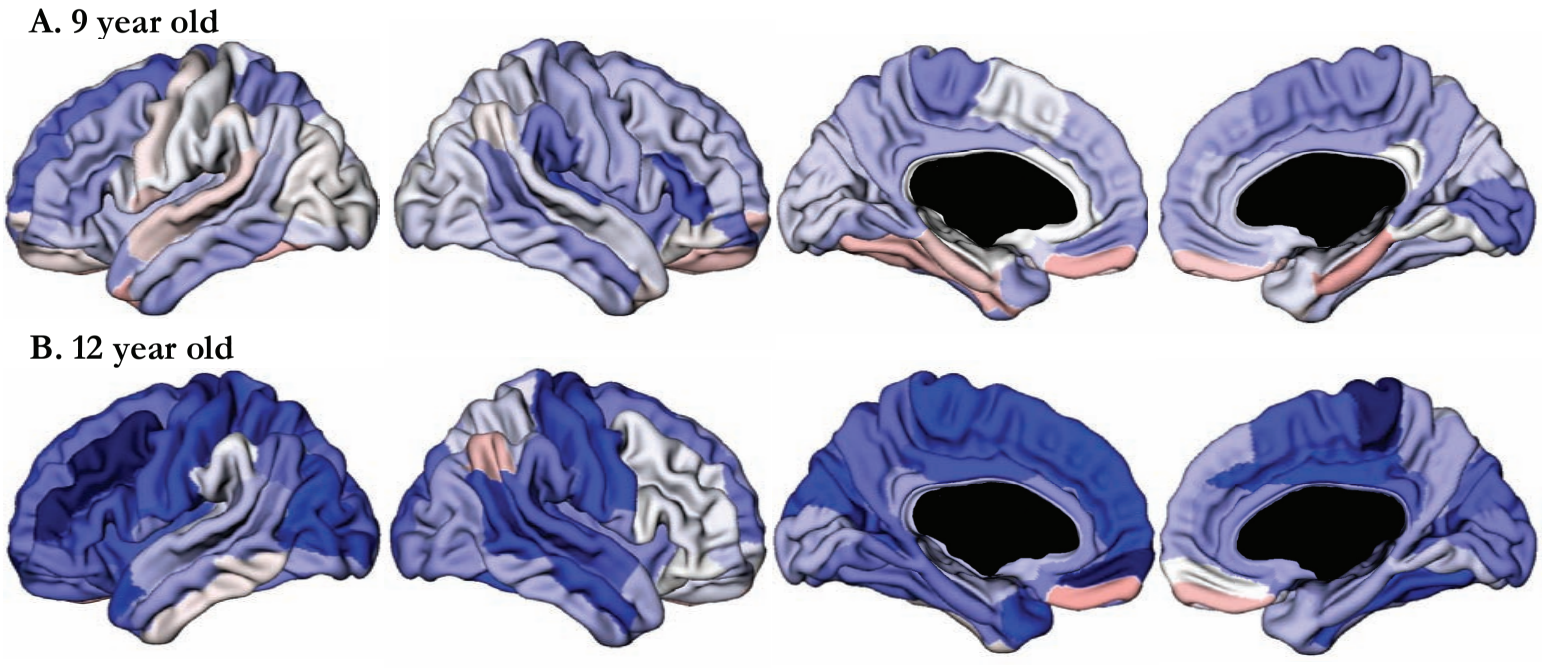

Figure 8.2 Phenotypic correlation (rp) between full scale IQ and cortical thickness (per region of interest) at age 9 (A) and 12 (B).

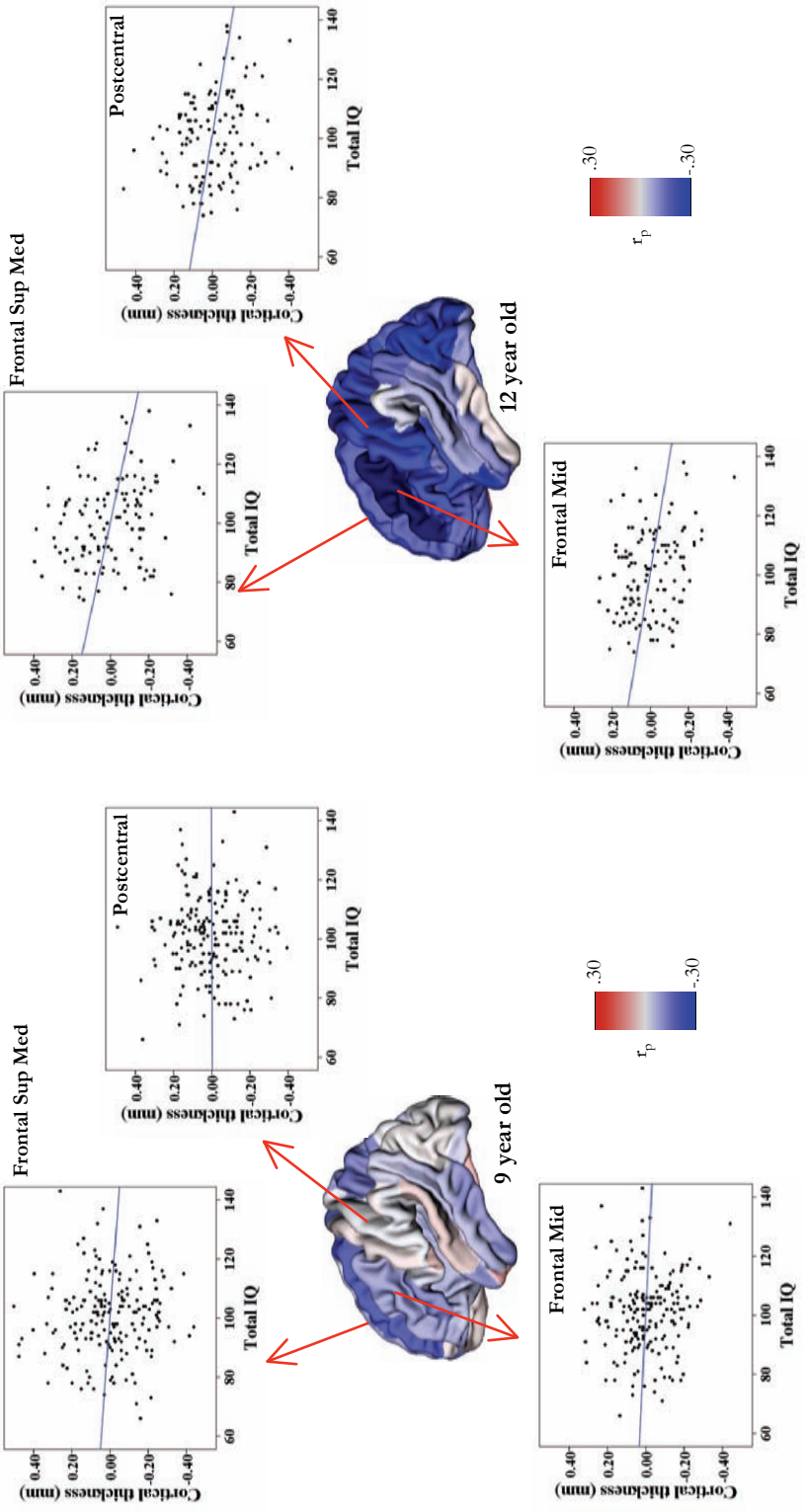

Figure 8.3 Scatter plots of the phenotypic correlation (rp) between total IQ and cortical thickness at ages 9 and 12 in left frontal superior medial, postcentral, and frontal middle cortical areas. Cortical thickness (mm) is corrected for age at scanning, sex, and handedness.

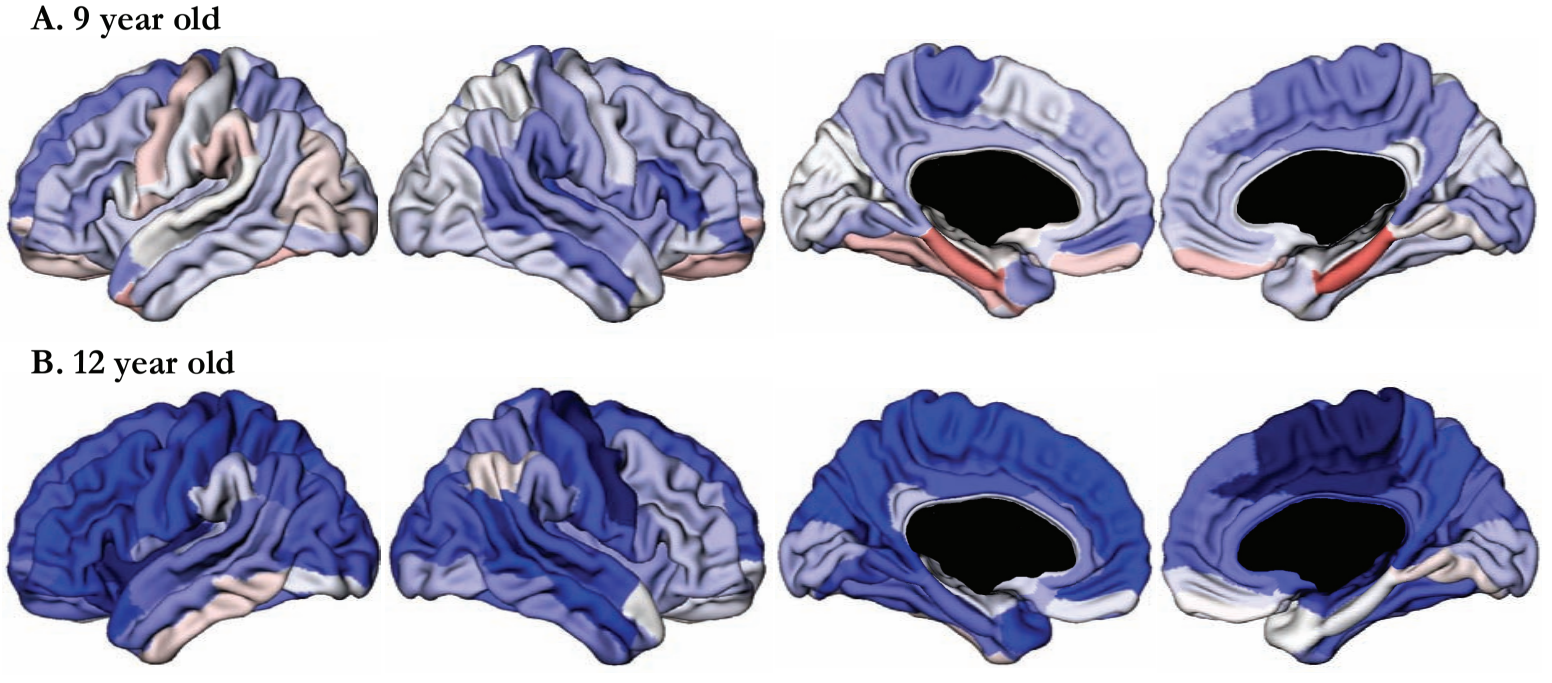

Figure 8.4 Phenotypic correlation (rp) between verbal IQ and cortical thickness (per region of interest) at age 9 (A) and 12 (B).

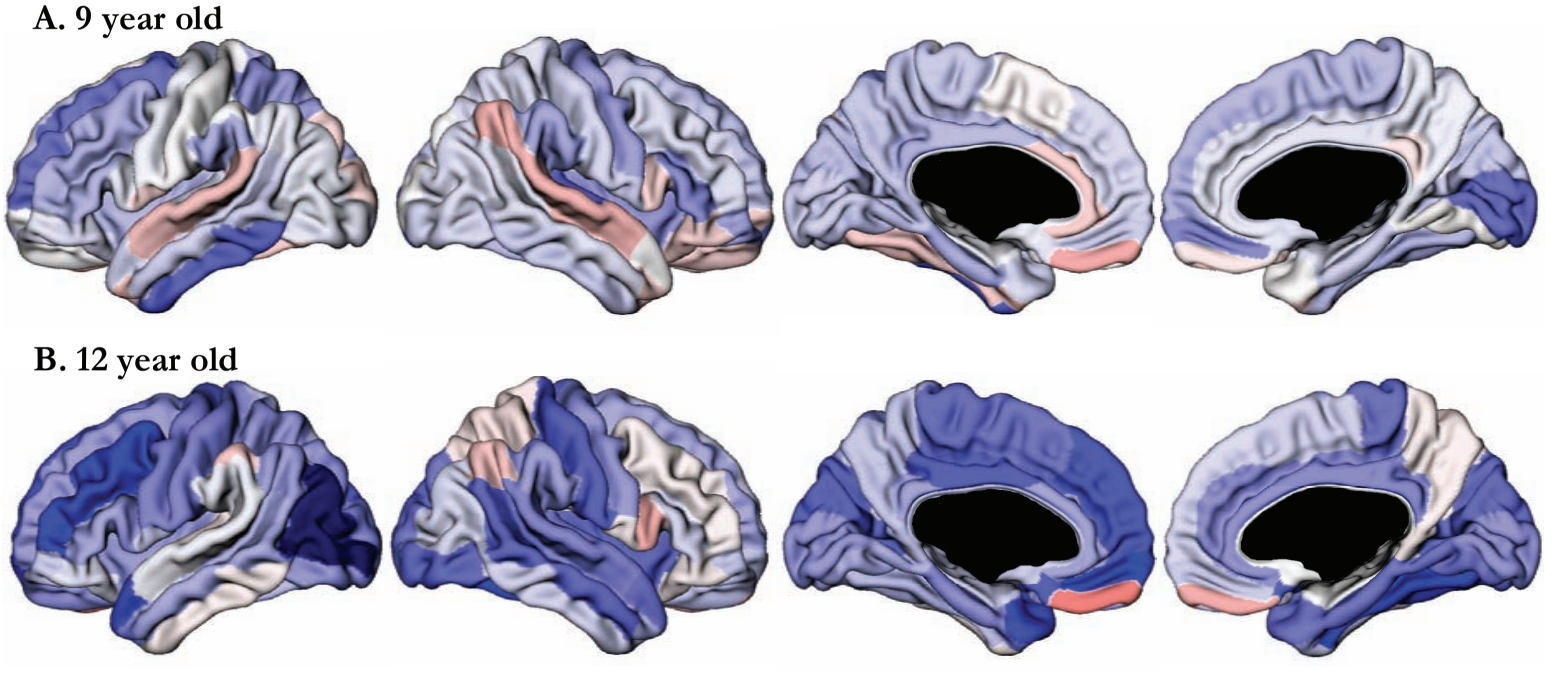

Figure 8.5 Phenotypic correlation (rp) between non verbal IQ and cortical thickness (per region of interest) at age 9 (A) and 12 (B).

Table 8.1 Phenotypic correlations (r_p) between full scale IQ and cortical thickness (ROI) at age 9 and 12 ($p < 0.05$).

		9 years old		12 years old	
	ROI	r_p	χ^2	r_p	χ^2
L	Precentral	0.02	*0.07*	**-0.22**	*4.92*
	Frontal Sup	**-0.19**	*5.73*	-0.17	*2.92*
	Frontal Mid	-0.09	*1.37*	**-0.30 ***	*9.34*
	Frontal Inf Oper	-0.03	*0.13*	**-0.24**	*5.94*
	Frontal Inf Tri	-0.12	*2.04*	**-0.20**	*4.16*
	Supp Motor Area	-0.02	*0.05*	**-0.25**	*6.19*
	Frontal Sup Medial	-0.08	*0.98*	**-0.28 ***	*8.19*
	Frontal Med Orb	-0.13	*2.63*	**-0.32 ***	*12.22*
	Insula	-0.07	*0.89*	**-0.23**	*5.69*
	Cingulum Mid	-0.10	*1.55*	**-0.23**	*5.04*
	Cuneus	-0.05	*0.39*	**-0.27 ***	*8.09*
	Occipital Mid	0.01	*0.02*	**-0.29**	*9.07*
	Postcentral	-0.01	*0.02*	**-0.27 ***	*7.48*
	Parietal Sup	-0.13	*2.44*	**-0.23**	*5.46*
	Paracentral Lobule	-0.14	*2.58*	**-0.29 ***	*7.70*
	Temporal Pole Sup	-0.09	*1.50*	**-0.29 ***	*9.55*
R	Precentral	-0.08	*1.09*	**-0.26**	*7.26*
	Frontal Mid Orb	**-0.15**	*3.91*	-0.06	*0.45*
	Frontal Inf Tri	**-0.20**	*6.69*	-0.02	*0.06*
	Supp Motor Area	-0.13	*2.27*	**-0.22**	*4.92*
	Cingulum Mid	-0.12	*2.03*	**-0.28 ***	*8.85*
	Cingulum Post	0.00	*0.00*	**-0.20**	*4.47*
	Calcarine	**-0.15**	*3.96*	-0.15	*2.51*
	Cuneus	-0.05	*0.38*	**-0.19**	*4.07*
	Fusiform	-0.07	*0.81*	**-0.26 ***	*8.15*
	Postcentral	-0.08	*1.00*	**-0.23**	*5.59*
	Paracentral Lobule	-0.13	*2.42*	**-0.31 ***	*10.14*
	Heshl	**-0.25**	*10.12*	**-0.21**	*5.15*
	Temporal Sup	-0.03	*0.10*	**-0.19**	*4.51*
	Temporal Mid	-0.12	*2.42*	**-0.25 ***	*8.23*

All analyses are corrected for sex, age at scanning, and handedness effects on cortical thickness; Full scale IQ was uncorrelated with mean cortical thickness at age 9 ($r = -0.11$), and was negatively correlated with mean cortical thickness at age 12 ($r = -0.27$); L = left side; R = right side; Bold = significant correlations at uncorrected level of significance ($p < 0.05$); * Significant FDR (0.05) corrected correlations, only present at age 12 (left side $\chi^2 = 7.48$, right side $\chi^2 = 8.15$).

Table 8.2 Phenotypic correlations (r_p) between verbal IQ and cortical thickness (ROI) at age 9 and 12 ($p < 0.05$).

		9 years old		12 years old	
	ROI	r_p	χ^2	r_p	χ^2
L	Precentral	0.05	*0.32*	**-0.23 ***	*6.52*
	Frontal Sup	**-0.16**	*4.11*	**-0.21**	*4.84*
	Frontal Mid	-0.07	*0.74*	**-0.28 ***	*8.18*
	Frontal Mid Orb	-0.09	*1.26*	**-0.21**	*5.03*
	Frontal Inf Oper	-0.02	*0.03*	**-0.27 ***	*7.67*
	Frontal Inf Tri	-0.11	*1.80*	**-0.26 ***	*7.37*
	Frontal Inf Orb	-0.06	*0.54*	**-0.28 ***	*10.08*
	Supp Motor Area	-0.03	*0.13*	**-0.27 ***	*7.14*
	Frontal Sup Medial	-0.08	*0.95*	**-0.29 ***	*9.67*
	Frontal Med Orb	**-0.15**	*4.29*	**-0.27 ***	*8.94*
	Insula	-0.06	*0.54*	**-0.30 ***	*11.30*
	Cingulum Mid	-0.09	*1.22*	**-0.21**	*4.75*
	Cuneus	0.00	*0.00*	**-0.28 ***	*9.47*
	Lingual	-0.09	*1.21*	**-0.20**	*5.38*
	Occipital Sup	-0.05	*0.85*	**-0.21**	*4.85*
	Occipital Mid	0.03	*0.12*	**-0.19**	*4.01*
	Postcentral	-0.02	*0.04*	**-0.30 ***	*9.93*
	Parietal Sup	-0.11	*1.70*	**-0.27 ***	*8.41*
	Angular	-0.06	*0.53*	**-0.19**	*3.84*
	Precuneus	-0.09	*1.04*	**-0.26 ***	*8.12*
	Paracentral Lobule	**-0.17**	*4.47*	**-0.29 ***	*8.57*
	Temporal Pole Sup	-0.12	*2.23*	**-0.27 ***	*8.77*
R	Precentral	-0.03	*0.18*	**-0.35 ***	*14.90*
	Frontal Sup	-0.07	*0.73*	**-0.22**	*5.20*
	Frontal Inf Tri	**-0.20**	*6.61*	-0.06	*0.49*
	Rolandic Oper	-0.13	*2.64*	**-0.20**	*3.99*
	Supp Motor Area	-0.15	*3.54*	**-0.40 ***	*18.15*
	Cingulum Mid	-0.14	*2.86*	**-0.39 ***	*19.21*
	Cingulum Post	-0.01	*0.72*	**-0.19**	*5.01*
	ParaHippocampal	**0.18**	*4.91*	-0.01	*0.10*
	Occipital Sup	-0.02	*0.06*	**-0.23**	*6.46*
	Occipital Mid	-0.03	*0.10*	**-0.20**	*4.82*
	Fusiform	-0.05	*0.41*	**-0.20**	*4.88*
	Postcentral	-0.09	*1.24*	**-0.23**	*5.76*
	Precuneus	-0.13	*2.40*	**-0.25**	*6.17*
	Paracentral Lobule	-0.15	*3.17*	**-0.36 ***	*12.81*
	Heschl	**-0.25**	*9.35*	**-0.20**	*5.15*
	Temporal Sup	-0.11	*1.68*	**-0.22**	*6.64*
	Temporal Mid	**-0.16**	*4.08*	**-0.29 ***	*11.99*

All analyses are corrected for sex, age at scanning, and handedness effects on cortical thickness; Verbal IQ was uncorrelated with mean cortical thickness at age 9 ($r = -0.10$), and was negatively correlated with mean cortical thickness at age 12 ($r = -0.32$); L = left side; R = right side; Bold = significant correlations at uncorrected level of significance ($p < 0.05$); * Significant FDR (0.05) corrected correlations, only present at age 12 (left side $\chi^2 = 6.51$, right side $\chi^2 = 11.99$).

Table 8.3 Phenotypic correlations (r_p) between nonverbal IQ and cortical thickness (ROI) at age 9 and 12 ($p < 0.05$).

		9 years old		12 years old	
	ROI	r_p	χ^2	r_p	χ^2
L	Frontal Sup	**-0.16**	*4.33*	-0.11	*1.26*
	Frontal Mid	-0.09	*1.35*	**-0.26**	*7.04*
	Olfactory	-0.03	*0.15*	**-0.19**	*3.91*
	Frontal Sup Medial	-0.04	*0.28*	**-0.21**	*4.04*
	Frontal Med Orb	-0.06	*0.53*	**-0.28**	*8.79*
	Occipital Mid	-0.02	*0.09*	**-0.33 ***	*11.78*
	Temporal Pole Sup	-0.04	*0.29*	**-0.25**	*6.31*
	Temporal Inf	**-0.18**	*5.68*	0.02	*0.03*
R	Calcarine	**-0.17**	4.92	-0.12	1.84
	Fusiform	-0.08	1.05	**-0.29**	9.19
	Heschl	**-0.18**	5.26	-0.14	2.08

All analyses are corrected for sex, age at scanning, and handedness effects on cortical thickness; Nonverbal IQ was uncorrelated with mean cortical thickness at both ages 9 ($r = -0.08$), and at age 12 ($r = -0.19$); L = left side; R = right side; Bold = significant correlations at uncorrected level of significance ($p < 0.05$); * Significant FDR (0.05) corrected correlations, only present at age 12 (left side $\chi^2 = 11.78$).

Genetic modelling

Heritability of full scale IQ was 40% at age 9 and 59% at age 12. Heritability for verbal IQ and nonverbal IQ was 37% and 60% at age 9, and 22% and 73% at age 12, respectively. For full scale and verbal IQ common environmental influences were estimated at 35% and 43% at age 9, and 22% and 52% at age 12 (see supplementary material; Table S1 and S2).

Results of the bivariate genetic model fitted on the data for the ROIs in which cortical thickness showed a correlation with full scale IQ, verbal and nonverbal IQ (FDR corrected; only present at age 12) are given in Table 8.4. In most ROIs in both left and right hemispheres, genetic correlations (r_g) were between -0.54 and -1.00, indicating a large or complete overlap between genetic factors acting on (verbal) IQ and the cortical thickness of that specific ROI.

Cortical gray matter volume and surface area

To get a better understanding on how other measures for cortical structure are related to the level of intelligence, the total cortical surface and cortical gray matter volume were calculated additionally and correlated with full scale IQ. Mean (s.d.) surface area of the whole cerebrum was 1828.2 (158.2) cm^2 at age 9, and 1854.2 (162.2) cm^2 at age 12. Mean cortical gray matter volume was 622.8 (54.5) ml and 616.1 (58.0) ml at ages 9 and 12. Phenotypic, genetic and environmental correlations between full scale IQ and cortical gray matter volume (ml), mean cortical thickness (mm), and cortical surface area (cm^2) are given in Table 8.5.

Table 8.4 Results of the bivariate genetic model for full, verbal and nonverbal IQ and ROI at age 12 (FDR corrected). Heritability (h^2) of cortical thickness (ROI), genetic (r_g) and environmental (r_e) correlations are given.

		ROI	r_p	r_g	χ^2	r_e	χ^2	h^2 ROI
Full IQ	**L**	Frontal Mid	**-0.30**	**-0.44**	*7.74*	0.10	*0.30*	0.62
		Frontal Sup Medial	**-0.28**	**-0.58**	*8.30*	0.17	*0.81*	0.53
		Frontal Med Orb	**-0.32**	**-1.00**	*11.35*	0.11	*0.38*	0.20
		Cuneus	**-0.27**	**-0.63**	*8.27*	0.23	*1.28*	0.49
		Postcentral	**-0.27**	-0.40	*3.59*	-0.17	*0.82*	0.49
		Paracentral Lobule	**-0.29**	**-0.32**	*4.83*	-0.24	*1.71*	0.77
		Temporal Pole Sup	**-0.29**	-0.57	*3.36*	-0.21	*1.41*	0.21
	R	Cingulum Mid	**-0.28**	-0.56	*3.59*	-0.15	*0.59*	0.24
		Fusiform	**-0.26**	**-0.55**	*3.89*	-0.07	*0.15*	0.25
		Paracentral Lobule	**-0.31**	**-0.60**	*5.69*	-0.13	*0.58*	0.33
		Temporal Mid	**-0.25**	-0.37	*2.09*	-0.20	*1.22*	0.30
Verbal IQ	**L**	Precentral	**-0.23**	-0.72	*2.43*	-0.04	*0.05*	0.33
		Frontal Mid	**-0.28**	**-1.00**	*14.16*	0.16	*0.81*	0.59
		Frontal Inf Oper	**-0.27**	-0.95	*3.03*	-0.14	*0.87*	0.20
		Frontal Inf Tri	**-0.26**	-0.60	*2.81*	-0.10	*0.27*	0.51
		Frontal Inf Orb	**-0.28**	**-1.00**	*3.96*	-0.06	*0.19*	0.19
		Supp Motor Area	**-0.27**	-0.61	*3.21*	-0.16	*0.85*	0.54
		Frontal Sup Medial	**-0.29**	**-1.00**	*7.91*	0.15	*0.79*	0.51
		Frontal Med Orb	**-0.27**	**-1.00**	*4.58*	0.06	*0.17*	0.21
		Insula	**-0.30**	-0.74	*3.83*	-0.05	*0.08*	0.35
		Cuneus	**-0.28**	**-1.00**	*11.61*	**0.39**	*6.37*	0.48
		Postcentral	**-0.30**	-0.56	*2.60*	-0.23	*1.54*	0.48
		Parietal Sup	**-0.27**	**-1.00**	*8.92*	0.15	*0.93*	0.38
		Precuneus	**-0.26**	**-1.00**	*7.63*	0.30	*3.20*	0.51
		Paracentral Lobule	**-0.29**	**-0.54**	*4.75*	-0.15	*0.56*	0.76
		Temporal Pole Sup	**-0.27**	-0.69	*1.15*	-0.21	*1.58*	0.19
	R	Precentral	**-0.35**	-0.77	*2.27*	-0.24	*2.22*	0.24
		Supp Motor Area	**-0.40**	**-0.59**	*4.02*	-0.31	*2.81*	0.46
		Cingulum Mid	**-0.39**	**-1.00**	*6.56*	-0.08	*0.20*	0.28
		Paracentral Lobule	**-0.36**	**-0.84**	*4.30*	-0.19	*1.32*	0.26
		Temporal Mid	**-0.29**	**-1.00**	*8.38*	0.07	*0.19*	0.34
Nonverbal IQ	**L**	Occipital Mid	**-0.33**	-0.38	*1.07*	0.02	*0.01*	0.51

L = left side; R = right side; Bold = significant correlations at level of $p < 0.05$.

Phenotypic correlations between full scale IQ and cortical gray matter volume at ages 9 and 12 were 0.38 ($p < 0.01$) and 0.22 ($p < 0.05$). Correlations between full scale IQ and cortical surface area at ages 9 and 12 were 0.29 / 0.32 (p's < 0.01). Thus, negative correlation can be found between IQ and mean cortical thickness at age 12, while positive correlations between IQ and surface area or cortical gray matter volume are observed at both ages. Total surface area and gray matter volume were highly correlated with each other at ages 9 and 12 ($r_p = 0.72$ / 0.69, p's < 0.01). Correlations between total cortical surface area and cortical thickness was -0.30 ($p < 0.01$) at age 9, and did not reached significance at age 12 ($r_p = -0.15$, $p = 0.15$).

Univariate genetic modelling revealed heritability estimates at ages 9 and 12 of 84% / 79% for gray matter volume, and 86% / 91% for total surface area (see supplementary material; Table S1 and S2). Bivariate genetic models were fitted to the data to explore whether genetic factors are shared between IQ and cortical gray matter volume or surface area. Genetic correlation at ages 9 and 12 between IQ and cortical gray matter volume were 0.52 ($p < 0.01$) / 0.38 ($p < 0.05$), and genetic correlations between IQ and surface area were 0.34 ($p < 0.05$) / 0.38 ($p < 0.05$; see Table 8.5).

Discussion

Cortical thinning occurring on the brink of puberty is accompanied by an increasing association with intelligence and this growing association is driven by genetic factors. At nine years of age, cortical thickness and intelligence are not significantly correlated. At age 12, an association emerges between intelligence and cortical thickness revealing that a thinner cortex is correlated with higher intelligence scores. When separating verbal and nonverbal IQ, it becomes apparent that this effect is mainly driven by the verbal counterpart of intelligence, showing correlations particularly in regions of the left frontal cortex. We found no significant associations between cortical thickness

Table 8.5 Phenotypic (above diagonal; r_p) and genetic (r_g) and environmental (r_e) correlations (below diagonal; r_g / r_e) between full scale IQ and cortical GM volume (ml), mean cortical thickness (mm), and cortical surface area (mm^2) at age 9 (A) and age 12 (B).

A. 9 year old

	Full scale IQ	GM volume	Mean CT	Surface area
Full scale IQ	-	**0.38**	-0.11	**0.29**
GM volume	**0.52** / -0.10	-	0.09	**0.72**
Mean CT	-0.12 / -0.18	0.17 / -0.14	-	**-0.30**
Surface area	**0.34** / 0.18	**0.79 / 0.36**	**-0.25 / -0.48**	-

B. 12 year old

	Full scale IQ	GM volume	Mean CT	Surface area
Full scale IQ	-	**0.22**	**-0.27**	**0.32**
GM volume	**0.38** / -0.22	-	**0.24**	**0.69**
Mean CT	**-0.33** / -0.05	**0.34** / -0.22	-	-0.15
Surface area	**0.38 /** 0.13	**0.74** / 0.25	-0.09 / **-0.60**	-

GM = gray matter; CT = cortical thickness; Significant genetic correlations ($p > 0.05$) are given in bold.

and nonverbal intelligence at this young age. A large to complete overlap between genetic factors acting on (verbal) IQ and the cortical thickness was observed. Thus, is seems that brain areas contributing to (verbal) intellectual performance are specializing with the onset of puberty, under the influences of overlapping genes between IQ and cortical thickness.

A higher verbal intelligence in 12-year olds was associated with a thinner cortex of the cerebrum. This finding is consistent with the observation that children having a higher intelligence show a steeper decline in cortical thickness during early adolescence (Shaw et al., 2006). Intriguingly, the negative correlations that we find here between cortical thickness and intelligence in adolescence seem in contrast to the positive correlations found between cortical thickness and intelligence in adulthood (Brans et al., 2010; Narr et al., 2007). We may argue that underlying mechanisms acting on cortical thickness during maturation are different from the mechanisms that act on cortical thickness in adults. Cortical thinning during puberty has been associated with the loss of unwanted connections (synaptic pruning; Huttenlocher and Dabholkar, 1997) and increased myelination of axons (white matter encroachment; Gogtay and Thompson, 2010). During aging, cortical thinning has been associated with decreases in dendritic spine numbers and density (Duan et al., 2003) and loss of synapses (Peters et al., 2008). It is not unlikely that a higher intelligence is associated with a faster cortical development in adolescence, but also acts as a defense mechanism against thinning in adult life.

In a cross-sectional sample of children aged 6 – 18 years a positive correlation between cortical thickness and general intelligence was observed by Karama et al. (2011). The difference between the 2 studies is puzzling and needs further exploration. There are some differences between the studies, which include IQ assessment, determination of cortical surface boundaries based on different gray and white matter segments, but these are not very large. More importantly, cortical development follows a non-linear pattern during puberty (Gogtay et al., 2004). It may be that the association between cortical thickness and intelligence changes across development. Thus, because of dynamic changes in the associations between cortical thickness and volume, surface area, and intelligence, the actual age of the sample, is crucial. Cortical thickness was corrected for age in the Karama study, but this approach may hide very specific age-dependent associations.

Our findings that correlations arise between cortical thickness and verbal intelligence is consistent with the correlations between left hemispheric cortical thinning and improvement on verbal tasks in young children (Sowell et al., 2004). Negative correlations between cortical thickness and verbal fluency have also been found in a cross-sectional sample of children aged between 9 and 16 (Porter et al., 2011). We may speculate that the different patterns that we observed between the ages of 9 and 12 result from the closure of a developmental window. It has been suggested that language acquisition occurs only under certain internal constraints, some of which undergo maturational change (Newport, 1990; Sakai, 2005). After the period in which language acquisition is easiest in children, the inborn capacity for verbal intelligence may manifest itself in the emergence of genetically driven associations between cortical thickness and verbal intelligence. Other evidence for this hypothesis is the fact that the same genes act on change in cortical thickness in both Broca's and Wernicke's areas in this sample (van Soelen et al., 2011b).

The most widespread and pronounced correlations between (verbal) intelligence and cortical thickness emerge in the (left) frontal lobe. Development of the frontal lobe has been linked to the development of cognitive function, specifically the development of temporal integration of information (Fuster, 2002), and might therefore not only be linked to language, but also to other forms of higher executive functioning. Our results may be viewed in the parieto-frontal integration theory (P-FIT) of intelligence (Jung and Haier, 2007) which links a network of areas (including dorsolateral prefrontal cortex, parietal lobe, anterior cingulate cortex, and regions within the temporal and occipital lobes) to cognitive abilities. This theory also emphasizes the left hemisphere as being the most important for cognitive task performance.

The negative correlations between cortical thickness and intelligence, arising between the ages of 9 and 12, are mainly driven by genes. As there is considerable thinning of the cortex in this age range which is genetically driven (van Soelen et al., 2011b), we hypothesize that the same genes are responsible for cognitive changes and cortical thinning at the start of puberty. In light of the positive associations between brain volumes and intelligence throughout the lifespan (McDaniel, 2005), which was also found in the present sample at childhood (van Leeuwen et al., 2009), we postulate that different sets of genes are involved in explaining the relations between intelligence with brain volumes, and intelligence with cortical thickness. We find genetic overlap between intelligence and cortical thickness and between intelligence and gray matter volume and cortical surface area. Cortical thickness and gray matter volume were (genetically) unrelated at age 9 and only partly overlapping at age 12. This is to some extent consistent with the finding that cortical thickness and surface area or cortical gray matter volume are driven by different genetic factors (Panizzon et al., 2009; Winkler et al., 2010). Total cortical surface area was relatively highly correlated with cortical gray matter volume, and both these measures of cortical structure were positively correlated with level of intelligence, while cortical thickness displayed a negative correlation. It is possible and indeed very likely that cortical thickness represents only one part of the puzzle linking structural brain development and intelligence throughout life.

The emergence of the relation between cortical thickness and intelligence, and more specifically with verbal intelligence at age 12 coincides with a changing etiology of full scale and verbal intelligence: around the start of puberty, genetic influences on full scale and verbal intelligence increase, while the environment becomes less important (Hoekstra et al., 2007; van Soelen et al., 2011c). The heritability estimates for IQ measures reported here are slightly different from those reported in van Soelen, et al. (2011c). That study represented a slightly larger sample in which siblings of twins were included. Here the main focus was to estimate cortical thickness and its relation to intelligence at a precise moment in development, and only included children in a very narrow age range, and hence no inclusion of siblings.

Even though the association between intelligence and brain morphology is genetically driven, finding candidate genes that cause this association is not straightforward. For example, the Met/Met variant of catechol-O-methyltransferase (COMT) is associated with a thicker cortex in adolescents (Shaw et al., 2009) and adults (Cerasa et al., 2010) and also with a higher cognitive functioning (e.g., Savitz et al., 2006). It must be noted effect sizes in this type of studies are usually very small. Brain-Derived Neurotrophic Factor (BDNF), is highly expressed in the cerebral cortex, and is implicated in synaptic plasticity (Cohen-Cory et al., 2010). The expression pattern of BDNF coincides with maturational timing of different anatomical regions of the cortex (Webster et al., 2002; Webster et al., 2006; Wong et al., 2009). BDNF has also been linked to cognitive abilities, but these studies are mainly conducted in elderly samples (e.g., Miyajima et al., 2008). It is likely that complex traits such as intelligence and brain morphology are influenced by many genes of small effect so that identification of specific genes remains difficult (Casey et al., 2010; Deary et al., 2009).

In conclusion, at 9 years of age the associations between cortical thickness and intelligence are limited. Three years later at age 12, a thinner cortex, mainly in the left hemisphere, is associated with higher verbal intelligence. These associations are driven by genes. Thus, is seems that brain areas contributing to (verbal) intellectual performance are specializing with at the onset of puberty under the influences of genes.

Table S.1 Twin correlations (and their 95% CI) for full scale, verbal, nonverbal IQ, cortical gray matter volume (ml), cortical thickness (mm), and surface area (mm^2).

	9 years		12 years	
	MZ	**DZ**	**MZ**	**DZ**
Full scale IQ	0.76 (0.63 – 0.84)	0.54 (0.34 – 0.69)	0.81 (0.69 – 0.88)	0.56 (0.35 – 0.70)
Verbal IQ	0.80 (0.70 – 0.87)	0.59 (0.40 – 0.73)	0.76 (0.61 – 0.85)	0.66 (0.48 – 0.78)
Nonverbal IQ	0.60 (0.41 – 0.73)	0.26 (0.00 – 0.48)	0.73 (0.57 – 0.83)	0.26 (-0.03 – 0.50)
GM volume	0.76 (0.59 – 0.87)	0.44 (0.17 – 0.63)	0.69 (0.44 – 0.87)	0.27 (-0.05 – 0.53)
Mean CT	0.62 (0.36 – 0.77)	0.38 (0.16 – 0.56)	0.80 (0.62 – 0.90)	0.27 (-0.07 – 0.55)
Surface area	0.79 (0.65 – 0.88)	0.42 (0.16 – 0.62)	0.89 (0.81 – 0.93)	0.40 (0.07 – 0.62)

MZ = monozygotic; DZ = dizygotic; GM = gray matter; CT = cortical thickness

Table S.2 Results of univariate genetic modelling for full scale, verbal, nonverbal IQ, gray matter volume (ml), mean cortical thickness (mm), and cortical surface area (mm^2). The proportion of variance (and their 95% CI) that could be attributed to genetic (A), common (C), and unique environment (E) are given.

	9 years			12 years		
	A	**C**	**E**	**A**	**C**	**E**
Full scale IQ	0.40 (0.04 – 0.80)	0.35 (0.00 – 0.64)	0.25 (0.17 – 0.39)	0.59 (0.21 – 0.87)	0.22 (0.00 – 0.55)	0.19 (0.12 – 0.32)
Verbal IQ	0.37 (0.07 – 0.74)	0.43 (0.06 – 0.68)	0.20 (0.13 – 0.32)	0.22 (0.00 – 0.64)	0.52 (0.13 – 0.76)	0.26 (0.16 – 0.42)
Nonverbal IQ	0.60 (0.06 – 0.73)	0.00 (0.00 – 0.45)	0.40 (0.27 – 0.58)	0.73 (0.40 – 0.83)	0.00 (0.00 – 0.27)	0.27 (0.17 – 0.44)
GM volume	0.84 (0.74 – 0.90)	-	0.16 (0.10 – 0.26)	0.80 (0.60 – 0.89)	-	0.20 (0.11 – 0.40)
Mean CT	0.65 (0.44 – 0.78)	-	0.35 (0.22 – 0.56)	0.82 (0.64 – 0.90)	-	0.18 (0.10 – 0.36)
Surface area	0.86 (0.77 – 0.91)	-	0.14 (0.09 – 0.23)	0.91 (0.81 – 0.95)	-	0.09 (0.05 – 0.19)

GM = gray matter; CT = cortical thickness

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9

Summary and Discussion

The aim of this thesis was to explore how genetic and environmental influences affect brain maturation and cognition around puberty in an epidemiological sample of children from the general population in the Netherlands. For this purpose, a large cohort of children was studied within a three year interval. The sample was recruited from the Netherlands Twin Register (NTR) and consisted of 112 families at baseline, including 9-year-old twins and an older sibling. There were 89 families (80%) who returned for the follow-up assessment. Data on cognitive abilities were collected with psychometric IQ tests and a comprehensive neuropsychological testing protocol. Brain anatomy was acquired by 1.5T magnetic resonance imaging (MRI).

9.1 Summary

The studies described in this thesis all include an epidemiological sample of children, aimed to obtain a population based sample from across the Netherlands (see **Chapter 2**). This thesis includes studies of genetic and environmental influences covering a broad range of aspects of structural brain maturation and cognition. See for an overview of the main findings Table 9.1. The aim of these studies was to gain insight into how events around birth can have an effect on brain development and cognition in later life, to describe the extent to which genetic factors influence individual differences in brain development and cognition and to study the relation between brain structure and cognition at ages 9 and 12 years. Its strength includes the large group of twins followed longitudinally with narrow age range at both assessments. This longitudinal, genetically informative design allows modeling of genetic influences across development. The key questions that were addressed are whether the same genes act across ages 9 and 12 years, or whether there are age-specific genetic factors that are expressed at one age but not at another, to what extent genetic and environmental influences act on structural brain changes between 9 and 12 years of age, and to what extent genetic and non-genetic influences on cognition and brain structure overlap. In this last chapter the main results of the study are summarized and their implications for further research are discussed.

Effects of premature birth

The aim of **chapter 3** was to assess the effects of gestational age (i.e. pregnancy duration) and birth weight on brain volumes and cognition at a later age. Nine-year-old twins (N = 192 twins) were included in this study with a gestational age between 32 and 40 weeks and within the normal range of birth weight, from 1525 to 3820 g. The main findings of this study were that shorter pregnancy duration was associated with a smaller cerebellar volume at the age of 9 years, when correcting for birth weight, gender, and age at scanning. A lower birth weight was also associated with lower intelligence quotient (IQ) at age 9, but this effect disappeared when intracranial volume differences were taken into account. This study, in a population-based sample of children, contributes to the understanding that long-term effects of preterm birth are not limited to the group of very preterm infants or infants born with very low birth weight.

Heritability of cognition

Chapter 4 and **chapter 5** focus on the influence of genetic factors on cognition throughout childhood and adolescence. In **chapter 4**, the genetic background of verbal learning, was studied in detail. The Dutch version of the Auditory Verbal Learning Test (AVLT) was used to assess individual

differences in dynamic measures of verbal learning ability in children. In this study 9-year old twins (N=112 twin pairs) and an older sibling between 10 and 14 years old (N = 99 individuals) were included. A list of 15 unrelated, concrete nouns was presented to the children by a neutral computerized voice over 5 identical trials. The children had to recall as many words as possible directly after each trial. Non-linear curves were then fitted for each child individually, resulting in two parameters; Learning Speed (LS), representing the proportion of verbal material not yet recalled in a previous trial that is recalled in a following trial; and Forgetting Speed (FS), representing the proportion of material that was successfully remembered previously, and that can no longer be recalled in a following trial. The main finding was that children improved with age in the capacity to add more new words to their phonological loop (reflected by an increase in LS). Differences in verbal learning abilities were moderately heritable in the 9-year olds (LS 43%; FS 20%), and in the 10-14 year olds (LS 43%; FS 30%).

For general cognitive abilities, as assessed by the Wechsler Intelligence Scale for Children (WISC), the stability is high: the longitudinal correlations between full scale intelligence scores (FSIQ), verbal (VIQ) and performance (non-verbal; PIQ) intelligence scores were .72, .72, and .56 between childhood (age 9-11 years), and early adolescence (age 12-14 years). In **chapter 5**, these longitudinal IQ data were analyzed in the twins and their siblings to answer the question to what extent heritability differed for FSIQ, VIQ, and PIQ between the two age groups. The main results were that there was an increase in heritability of all intelligence scales during the transition from childhood to early adolescence. Heritability of FSIQ increased from 34% in childhood to 65% in early adolescence. Common environmental influences on the other hand, decreased with age for FSIQ, namely from 43% in childhood down to 18% in early adolescence. For VIQ a similar pattern of genetic and environmental influences was observed. Heritability increased from 37% in childhood up to 51% in adolescence, while the contribution of common environmental factors decreased from 42% down to 26%. For PIQ, common environment influences did not reach significance for PIQ in both age groups. Heritability was 64% in childhood and 72% in early adolescence. Next, the causes of stability were explored. The stability of FSIQ and VIQ across time was explained by genetic and to a smaller extent by common environmental influences, while stability of PIQ was completely explained by genetic influences. The results of this chapter confirm the robust findings of increased heritability of general cognitive abilities during the transition from childhood to adolescence, and the differences found between verbal and performance IQ suggest that these findings verbal and nonverbal domains have different developmental trajectories.

Heritability of structural brain changes

In **chapter 6** and **chapter 7**, genetic influences on structural brain changes were explored. In **chapter 6,** the extent to which genes influence volumetric brain measures at age 9 (N = 190 twins) and 12 (N = 125 twins), and volumetric changes between age 9 and 12 was investigated. An additional question was whether brain volume changes might reflect the strong increase in height that occurs in children at the beginning of puberty, or whether different processes, controlled by other genes are involved. The main findings were that at age 9 and 12, height and brain volumes were highly heritable. Heritability estimates at age 9 and 12 were high for height (93% / 93%), total brain (93% / 96%), total cerebral (93% / 96%), total cerebellar (95% / 95%), cerebral gray matter (88% / 92%), cerebral white matter (88% / 88%), and cerebellar gray matter (93% / 80%) volume. Heritability of lateral ventricle volume was (80% / 75%), and reached (61% / 59%) for third ventricle volume. Somewhat lower heritability estimations were observed for the cerebellar white matter volume (64 % / 49 %). For all brain volumes high genetic correlations over time were observed, indicating that individual variation in these brain volumes at the ages of 9 and 12 years were completely explained by the same genetic factors.

Between the age of 9 and 12, there were volumetric brain changes. Total brain volume increased on average with 14.6 ml, which is an increase of 1.1% change from baseline. The most pronounced volumetric change was observed for the cerebellum (increase of 4.2%). Cerebral gray matter volume was the only volumetric measure that showed a decrease in volume between 9 and 12 years of age (decrease of 1.6%). Individual differences in volumetric brain changes were partly under genetic control (change in cerebral volume was 20% heritable and in cerebellar volume 45%). The association between changes in cerebellar and cerebral volumes (r_p = 0.49) was driven by shared genetic influences and, to a smaller extent, by shared unique environmental influences. Thirdly, the change in height was highly heritable (73%), but was influenced by other genetic factors than the genetic factors implicated in changes in cerebral volume. What little was shared between height and cerebellar growth (r_p = 0.24), could be attributed partly to shared genetic influences (r_g = 0.48). We can conclude that we find developmental brain changes in this three year interval, as reported earlier over larger age spans in children and adolescents (Giedd et al., 1999), and that these changes are heritable. Furthermore, these changes in cerebral volume are not shared with the increase seen in height, while the change in cerebellar volume is partly (genetically) correlated with changes in height.

Overall, cerebral gray matter volume decreased during the three years period that the sample was followed. Locally this decrease in gray matter volume was associated with cortical thinning during adolescence. **Chapter 7** describes to what extent genetic influences affect the individual differences in cortical thickness changes across time or across regions. In the same twin sample as described in **chapter 6**, the thickness of the cortex was computed across the cerebrum at age 9 and at age 12. The main findings were that at age 9, several areas of the cortex showed significant heritability estimates ranging up to 78% in the left and up to 73% in the right hemisphere. Areas that showed significant contributions of genetic influences at age 9 were located bilaterally in frontal, midsagittal frontal, temporal and inferior parietal areas, inferior and medial insula, cingulate, the anterior paracentral lobule, lingual gyrus, precuneus, cuneus, calcarine sulcus, lateral occipital areas, and left parahippocampal gyrus. At age 12, heritability estimates were up to 88% and 91% in left and right hemispheres, respectively. Significant heritability estimates were located bilaterally in the precuneus, left inferior, and midsagittal superior frontal areas, left anterior paracentral lobule, left inferior temporal, and left lateral occipital gyri. It the right hemisphere, significant heritability estimates were found in the superior frontal, inferior parietal, inferior temporal, and cuneus.

Considerable thinning of the cortex was observed within the three year interval, most pronounced in the frontal pole and orbitofrontal, sensory-motor and visual cortices. This process was heritable (locally up to 79%), and the degree of genetic influence differs for the various areas of the brain. The same genetic factor operates in language areas (e.g., left inferior frontal (superior of) Broca's and left parietal (superior of) Wernicke's area). In these areas amplification of the same genetic factors across ages 9 and 12 was observed. Between these two regions, the genetic factors acting on cortical thinning were completely overlapping. This factor was independent from the genetic factor influencing left anterior paracentral (sensory motor) cortical thinning. Cortical thinning in the left and right frontal poles is driven by two genetic factors: one factor of decreasing influence over time, and another independent genetic factor at age 12 in left and right frontal poles. Thus, developmental thinning of the cerebral cortex in children and adolescents (Gogtay et al., 2004) is heritable in children between ages 9 and 12. Different genetic factors are responsible for variation in cortical thickness at ages 9 and 12, with independent genetic factors acting on cortical thickness across time and between various brain areas.

To summarize the studies in **chapter 6** and **7,** it is evident that the brain undergoes dramatic structural brain changes, now shown for the first time in a specific small time window at the

onset of puberty. Overall, brain volumes increased, and only cerebral gray matter volume decreased (**chapter 6**). The gray matter volume decrease was accompanied by thinning of the cerebral cortex, which differed in magnitude across different regions of the cerebrum (**chapter 7**). The areas that contained the most pronounced thinning were in the regions of the frontal pole and orbito-frontal, sensory-motor and visual cortices, and partly overlapped with previous findings of a time-region specific pattern of cortical maturation (Gogtay et al., 2004; Sowell et al., 2002). We found that changes in brain volumes and local cortical thickness are under genetic control.

Association between brain structure and cognition

In **chapter 8**, cortical thickness was computed for 78 regions of interest (ROIs) for all 9 and 12 year old twins with available MRI data (total N = 315 twins; as described previously in **chapter 6** and **7**). Full scale, verbal and nonverbal IQ (previously described in **chapter 5**) were correlated with cortical thickness (ROIs) at both ages. At age 9 the correlation between cortical thickness and IQ was small and not significant. At age 12, an association emerged between IQ and cortical thickness, revealing that a thinner cortex was correlated with a higher IQ. Phenotypic correlations reached up to -0.32. The observed correlation between IQ and cortical thickness, was mainly driven by verbal IQ and particularly so in the left frontal cortex (correlations of -0.21 up to -0.29). The associations between (verbal) IQ and cortical thickness were largely explained by shared genetic factors between IQ and cortical thickness.

Cortical thickness and gray matter volume were (genetically) unrelated at age 9 ($r_p = 0.09$) and only moderately correlated at age 12 ($r_p = 0.24$; $r_g = 0.34$). Total cortical surface area was relatively highly correlated with cortical gray matter volume at age 9 ($r_p = 0.72$; $r_g = 0.79$; $r_e = 0.36$) and at age 12 ($r_p = 0.69$; $r_g = 0.74$). Intelligence was positively correlated with cortical gray matter volume at age 9 ($r_p = 0.38$; $r_g = 0.52$) and at age 12 ($r_p = 0.22$; $r_g = 0.38$). Also cortical surface area was positively correlated with intelligence at age 9 ($r_p = 0.29$; $r_g = 0.34$) and at age 12 ($r_p = 0.32$; $r_g = 0.38$). Thus, both cortical gray matter volume and surface area were positively correlated with level of intelligence, while cortical thickness displayed a negative correlation. From this chapter it can be concluded that brain areas contributing to verbal intellectual performance are specializing with the onset of puberty under the influences of overlapping genes between verbal IQ and cortical thickness.

9.2 General discussion

Children undergo considerable changes in brain structure and function during the transition from childhood into adolescence. To get a better understanding of why individual differences in healthy brain development arise, using twin studies it is possible to explore to what extent genetic and environmental factors explain individual differences in brain development and cognition. This thesis has brought together a series of studies trying to get a more comprehensive view on the normative brain development by looking at the effects of birth parameters on later brain volumes and cognition at a later age, and genetic and environmental influences on different aspects of brain structure and cognition around puberty. How these results should be viewed in the light of current knowledge on healthy development, and developmental models of psychopathology and what their possible implications for further developmental studies are, will be discussed in the last part of this thesis.

Healthy and disrupted brain development

Very preterm birth has profound effects on brain development in infants (see for review, Hart et al., 2008; Weindling, 2010), but also in adolescence (Skranes et al., 2005; Allin et al., 2001; Parker et al., 2008). The cerebellum is under rapid development in the last trimester of the pregnancy (Limperopoulos et al., 2005), and therefore the development of this structure in postnatal life might be particularly vulnerable to preterm birth (**chapter 3**). Interestingly, the association between gestational age and cerebellar volume was absent at the age of 12 years. The relatively large increase in cerebellar volume observed between age 9 and 12 (**chapter 6**) is consistent with previous findings that the cerebellum is maturing at a later age than the cerebrum (Tiemeier et al., 2010). It can also be speculated that in this sample, the relatively large increase in cerebellar volume is partly compensating the effects of preterm birth that were found at age 9.

Since the majority of psychiatric disorders emerge during adolescence (Shaw et al., 2010; Gogtay et al., 2002), understanding the relative influences of genetic and environmental effects on brain development during this crucial period may elucidate biological processes underlying these illnesses. Why an individual develops a psychiatric disorder or not is most likely a result of interacting mechanisms of genetic predisposition and environmental factors that a person can encounter in early or later in life. The neurodevelopmental model for schizophrenia assumes that brain development is disturbed at a critical moment, which will lead to a predisposition, or a vulnerable state of the brain, already in an early stage in life (Weinberger, et al., 1987; Murray & Lewis, 1987). When first episodes of psychosis become apparent in late adolescence or early adulthood, this can be a result of compensatory systems no longer being sufficient to handle underlying disrupted processes (Thompson and Levitt, 2010). In combination with the extensive brain changes during puberty it might be that the brain is more sensitive or vulnerable to environmental factors, that could determine whether or not a person will develop schizophrenia (e.g., Insel, 2010). However, full siblings of childhood-onset schizophrenia (COS) patients exhibited gray matter deficits at a young age, but did not eventually progress into the gray matter deficits that were observed in their affected siblings in adolescence (Gogtay et al., 2007). In fact, at the age of 18-20 years, gray matter deficits found in COS patients, were no longer present in full siblings who ultimately remained healthy (Gogtay et al., 2007). The authors state that the exact causal mechanisms remain unknown (Gogtay and Thompson, 2010), but it seems that although these subjects have a (genetic) predisposition, they also have some protection for the actual development of the psychiatric diagnosis. This further highlights the complex interplay between genetic and environmental influences and development.

It is important to realise that cross-sectional studies, where group level differences are explored (e.g. between different age groups, or comparing pediatric with adult samples), are not informative regarding the developmental trajectory of the individual brains (Karmiloff-Smith, 2010). Because of this, longitudinal studies are of great importance. Using a developmental approach, i.e. mapping cortical gray matter changes in normal development can create opportunities for modelling disrupted trajectories in psychopathology (Gogtay and Thompson, 2010). Gray matter development in childhood ADHD seems to be associated with cortical developmental curves that show similar typical characteristics as healthy trajectories, but seems to be shifted along the age axis, resulting in a delayed cortical maturation (Shaw et al., 2007a). In COS, developmental trajectories seem to be characterized by differences in the velocity of developmental changes, i.e. the basic shape of neurodevelopmental curves remains intact, but with disrupted tempo (Arango et al., 2008; Thompson et al., 2001).

How should we map the developing brain?

When exploring brain development, there are different methods to map structural brain changes. In this thesis the main focus was on brain volumes and the thickness of the cortex. Although it seems logical that the product of cortical thickness and cortical surface areas gives the resulting cortical gray matter volume, this does not automatically imply that cortical thickness and volumetric measures of gray matter tap into the same underlying construct. Indeed, as reported in this thesis cortical gray matter volume was uncorrelated with the thickness of the cortex at age 9, and only to a small extent correlated with each other at age 12 (**chapter 8**). Cortical gray matter volume was strongly correlated with cortical surface (Schnack, et al, *in preparation*; **chapter 8**). These finding are in line with previous studies in adult samples, where cortical surface was uncorrelated with cortical thickness (Panizzon et al., 2009). Another study that replicated this finding included volume measures as well, confirming that cortical surface is indeed more related to cortical volume than to cortical thickness (Winkler et al., 2010). The surface area is also dependent on factors that reflect the shape of the cortex, e.g. cortical folding or the amount of gyrification. Previous studies reported that surface area is increasing in childhood (Sowell et al., 2002), and also changes in gyrification during adolescence are observed (White et al., 2010). In addition, the finding that IQ is correlated negatively with cortical thickness at age 12, while a positive correlation exist between IQ and gray matter volume at the same age (**chapter 8**), creates room to discuss how these measures of structure are capturing different concepts of the developing cerebral cortex.

Imaging techniques like magnetic resonance imaging are limited in exploring the actual changes at neuronal and cellular levels (e.g., dendrites, spines, synapse connections). The cortex is shaped by neurons that are parallel aligned in different lamina (cortical layers), and the composition of these layers differs across the cortex. The underlying biological mechanisms that cause changes in the cortex like increasing surface or thinning of the cortex remains under debate (e.g., decrease of gray matter or increase of myelination). The same accounts for the developmental changes of cortical folding or gyrification during adolescence (White et al., 2010). This illustrates the importance of combining different morphological measures to get a more comprehensive view on individual differences in structural brain development. Maybe a specific measure of brain structure characterizes a disorder, which would not be detected using other measures. For instance, in a study of aging, differences in structural changes in the medial temporal lobe were explored between healthy aging and Alzheimer patients. Decreased volumes and surface area were observed in both groups, but in de Alzheimer patients, stronger thinning of the cortex was also observed (Dickerson et al., 2009). In adults with autism, age was found to be associated with a thicker cortex, but not with greater surface area (Raznahan et al., 2010).

Development in cognitive function

The period of childhood to adolescence is characterized by cognitive maturation (Casey et al., 2005b;Keating, 2004;Steinberg, 2005). Children improve considerably in specific cognitive functions, such as information processing (Demetriou et al., 2002), working memory (Gathercole et al., 2004), and verbal learning (van den Burg and Kingma, 1999). In **chapter 4**, the older participants (siblings of 9-year olds twins) had higher LS scores, meaning that they had a steeper learning curve, reaching a higher number of new words that they could remember in a fewer numbers of trials. There were no other specific cognitive tasks included in this thesis exploring increased performance within different cognitive domains, possibly in combination with structure brain measures (e.g., short term and long term memory, working memory, verbal fluency, and more). It was clear that all subjects increased their performance with age on the cognitive tasks included in the neuropsychological testing protocol, as reported in **chapter 2** (Table 2.3).

Genetic influences on brain development and cognition

Genetic influences that act on developmental changes between age 9 and 12 are of major significance. In **chapter 4**, the genetic contribution on parameters that described the learning curve of performance on a verbal learning task in childhood was reported. Cognitive abilities can be an indicator of psychiatric disorders later in life (Woodberry et al., 2008; Reichenberg et al., 2010; van Oel et al., 2002). More specifically, memory impairments are one of the most commonly found cognitive deficits in patients with schizophrenia (Toulopoulou et al., 2003). A recent study including a large sample of adult patients with schizophrenia showed that they performed worse on several aspects of the Rey's auditory verbal learning task compared to healthy age and IQ matched controls (Badcock et al., 2011). Also in healthy relatives of patients with schizophrenia, decreased memory functioning has been reported, confirming the association between genetic influences on memory functioning and the genetic susceptibility to schizophrenia (see for review, Reichenberg and Harvey, 2007). Genetic overlap was indeed found on verbal learning performance and schizophrenia liability (Owens et al., 2011; Toulopoulou et al., 2010). However, heritability on a specific trait or psychiatric disease is no guarantee for success in genome wide association studies (Manolio et al., 2009; van Haren et al., 2008). If dynamic measures of verbal memory (**chapter 4**) could act as an intermediate phenotype for schizophrenia should be further explored in future research (Gur et al., 2007; de Geus et al., 2001).

General cognitive ability is stable across life, and the increase in genetic influences with increasing age, together with decreasing common environmental influences, is a common finding (Deary et al., 2009; Davis et al., 2009; Haworth et al., 2010). Longitudinal twin studies have generally found that variation in IQ is explained by the same genetic factors at different ages and in in this thesis, an increase in heritability across the different IQ scales was also reported (Davis et al., 2008; Bartels et al., 2002; Davis et al., 2009; Lyons et al., 2009; Boomsma and van Baal, 1998). The increase in heritability of intelligence may be the result of several processes. Genetic amplification has been suggested as the most likely explanation (DeFries et al., 1987), and this is what was observed in **chapter 5**. As children grow older they are more likely to select or even create their own environment, driven by their genetic disposition, resulting in an increased expression of their genetic potential (Plomin et al., 1977) and this explanation is compatible with the amplification hypothesis. Simultaneously, common environmental influences that are present in childhood diminish with increasing age. The increase of genetic and decrease of common environmental influences on IQ could be a result of children becoming more independent of their familial environmental and parental influences (Scarr and Mccartney, 1983). The increase of genetic and decrease of common environmental influences on IQ was mainly driven by the verbal counterpart of intelligence, and not by nonverbal abilities (**chapter 5**), in line with previous findings in an independent Dutch sample (Hoekstra et al., 2007). An interpretation of common environmental influences acting on verbal IQ in childhood and early adolescence can be for instance socioeconomic status (SES). SES is a measure of one's overall status and position in society, and has effects on cognition, academic, achievement and mental health (see for review, Hackman et al., 2010), and is assumed to be similar for family members. A family's SES has been increasingly recognized as an important influence on the development of children (Hackman and Farah, 2009). More specifically, SES has been implicated in explaining individual differences in vocabulary, phonological awareness and processing, and syntax in children (Whitehurt, 1997), which is under strong developmental changes up to puberty (Sakai, 2005).

Previous studies have indicated that the genetic influences acting on brain morphology overlap with genetic influences on intelligence in adults (Posthuma et al., 2000; Hulshoff Pol et al., 2006), and in childhood and adolescence (van Leeuwen et al., 2009; Betjemann et al., 2010; Wallace et al.,

2010). The correlation between cortical thickness and IQ at age 12 (see **chapter 8**), was largely explained by shared genes between IQ and cortical thickness. The emergence of the relation between cortical thickness and intelligence, and more specifically with verbal intelligence at age 12 coincides with a changing etiology of full scale and verbal intelligence: around the start of puberty, genetic influences on full scale and verbal intelligence increase, while the environment becomes less important (Hoekstra et al., 2007; **chapter 5**).

Other studies have reported high heritabilities for aspects of brain anatomy, assessed by different imaging techniques in children and adults. Different brain volumes and the microstructural properties of white matter are heritable (Peper et al., 2007; Brouwer et al., 2010; Peper et al., 2009; Wallace et al., 2006; Baare et al., 2001). Heritability for local cortical thickness throughout the cortex was previously reported in children (Yoon et al., 2010; Lenroot et al., 2009), adults (Brans et al., 2010), and for a region of interest approach in middle-aged men (Kremen et al., 2010; Panizzon et al., 2009). This thesis now described for the first time that genes affect individual differences in brain changes at onset of puberty.

Concerning the heritability on global volumetric changes; genetic factors acting on volumes at age 9 were completely overlapping with the genetic factors acting on volumetric changes between 9 and 12 years of age (**chapter 6**). In adults, there was evidence for distinct genetic factors acting on brain volumes and for genetic factors acting on volumetric changes (Brans, 2009). For local cortical thickness there were regions where increased or decreased influence of the same genetic factors acting at age 9 and 12 were observed (**chapter 7**). In addition, regions were identified with independent genetic factors at age 9 and 12, possibly reflecting specific genetic influences emerging for developmental processes in the cortex at the onset of puberty. This was partly similar to findings in adults, where local cortical thickness and the change in cortical thickness with increasing age was explored, and where also different genetic factors acted on change and on cortical thickness itself (Brans et al., 2010). These regions did not overlap between adults and children however.

Thus, when genetic influences on global volumes are explored, amplification (also observed for IQ) of the same genetic factor across time is observed, while for local thickness of the cortex other genetic mechanisms are observed over time. These differences between adults and children emphasize further that brain changes in adolescence have different underlying mechanisms or are for a different purpose, (i.e., maturation of the brain), than brain changes observed in adulthood.

In a large cross-sectional pediatric study with a wide age range of 5 up 19 years of age, Schmitt et al., reported that regions that were functionally or anatomically connected with each other were more likely to be under control of the same genetic factors (Schmitt et al., 2008). Results from this thesis now showed that this phenomenon might also hold for genes acting on the amount of thinning of the cortex (**chapter 7**).

Underlying genetic mechanisms and candidate genes

Searching for the actual genes that are involved in healthy brain development can help us to understand the genetic basis of disorders that are characterized by disrupted brain development or impaired cognitive functioning. For instance, many of the risk alleles that are associated with schizophrenia have been found to be involved in developmental processes (Walsh et al., 2008; Nakata et al., 2009; Colantuoni et al., 2008). There are many underlying biological processes that affect a phenotype such as brain structure or the complex trait of intelligence, which in turn involves probably a large set of genes, although each single gene might only represent a small proportion of the complete variance of a phenotype.

We measured changing genetic influences on cortical brain development between 9 and 12 years of age. It is likely that more age-specific genetic factors come into play at specific points in the maturation process when children go through later stages of puberty. The search for genes associated with the developing brain becomes more challenging when considering that genes can change their expression patterns across time, which is likely to be linked to specific developmental stages or brain regions. Different forms of gene and environment correlations or the presence of gene by environment interactions can arise or change throughout life, especially in the life period of entering adolescence.

Gene-environment correlation occurs when genetic factors influence the person's exposure to the environment. Gene by environment interaction is about the genetic sensitivity or susceptibility to specific environments. At this moment, gene-environment interaction is getting more attention in different fields on research and different psychiatric disorders (e.g., Thapar et al., 2007; Hodgins-Davis and Townsend, 2009; Plomp et al., 2009). It is important to be aware of the consequences of correlation or interaction when they are not explicitly modelled (e.g., Purcell, 2002). When genetic variance is correlated with common environment, this will result in increased common environmental variance (i.e., passive; heritage of genes by parents and the home environment), or when the correlation is linked to unique environmental factors this can result in increased unique environment (i.e., evocative; based on genotype, individuals can attract specific environment to themselves, or active; individuals seek or create their own environment based on their genetic preposition). When gene-by-environment interaction is present it will increase the unique environmental variance if the interaction is with unique environment, if the interaction is with common environment, then the variance of the interaction term is included with the genetic variance. Genetic and environment correlation has been hypothesized previously in the case for IQ when children grow up. Changes in expression patterns of genes or the interaction with environmental factors can be one explanation for the changes in heritability, amplification of genetic variance (observed on measures of IQ, brain volumes and cortical thickness changes), or the independent genetic factors observed for local cortical thickness.

Finding the actual genes that are associated with developmental brain changes and / or are linked with cognitive functions is not straightforward. In the last few years, genome-wide-association studies were the dominating approach to find specific genes for wide variety of traits. For psychiatric disorders some findings have started to emerge (e.g., for schizophrenia and bipolar disorder). However, the number of individuals included in consortium based meta-analyses of cognition, psychological or psychiatric phenotypes does not yet approach the numbers that have been analyzed in genome-wide-association studies of e.g. height (Allen et al., 2010), BMI (Speliotes et al., 2010), blood lipids (Teslovich et al., 2010), and other metabolic traits for example (e.g., Manolio et al., 2009).

Some genetic variants have been associated with disorders where cognitive functioning is in some way affected (Flint, 1999; Deary et al., 2009). For most imaging genetics studies that are now reported a candidate gene approach is used, instead of a whole genome search, although first attempts to conquer the statistical challenges by collaboration between different imaging laboratories are on the way (Thompson and Martin; Shen et al., 2010; Stein et al., 2010). A candidate gene that is of interest for the developing brain is for example the Brain-Derived Neurotrophic Factor (BDNF; Cohen-Cory et al., 2010)). This gene is highly expressed in the cerebral cortex, and has an important role during brain development and in synaptic plasticity (Cohen-Cory et al., 2010), and has been associated with prefrontal cortex and hippocampal volume in healthy individuals (Pezawas et al., 2004). The expression pattern of BDNF is peaking around adolescence, was found to be unique for different anatomical regions, and coincides with maturational timing of different anatomical regions of the cortex (Webster et al., 2002; Webster et al., 2006; Wong et al., 2009). BDNF has also

been linked to cognitive abilities, but these studies are mainly conducted in elderly samples (e.g., Miyajima et al., 2008). Changes in expression levels in the prefrontal cortex were also found for dopamine receptors (DAR1), $GABA_A$ receptor alpha-subunits, and Apolopoprotein-D (Duncan et al., 2010; Kim et al., 2009; Weickert et al., 2007). Another example is the Met/Met variant of catechol-O-methyltransferase (COMT) that was previously associated with a thicker cortex in adolescents (Shaw et al., 2009) and adults (Cerasa et al., 2010), and also with a higher cognitive functioning (e.g., Savitz et al., 2006). Furthermore, the Epsilon 4 allele of the Apolipoprotein gene (APOE), which is commonly known to be associated with Alzheimer disease, was not only associated with gray and white matter reduction in elderly, but also with an altered brain developmental trajectory in children, as well as the resting brain activity in young adulthood (Shaw et al., 2007b).

These are only a few examples of candidate gene studies, and how their results should be viewed in combination with environmental factors is unclear. The search for the actual genes that are involved in developmental changes of the anatomy and function of the brain is challenging (Casey et al., 2010). Trying to find the link between specific genes and brain structure and brain function (and eventually behaviour) remains difficult considering the fact that they are end products of the combination of genotype, environment, and development. Genetic effects are not static throughout life, or between individuals, but emerge from dynamic processes like changing environmental during different developmental periods (see for more discussion; Casey et al., 2010).

9.3 Future directions

Each new step forward in research will automatically raise new questions as well. Next to biological changes during adolescence, emotional and motivational changes occur, which in turn could influence behavioral tendencies (Forbes and Dahl, 2010). Therefore, the possible role of environment (i.e., SES; Hackman and Farah, 2009;Hackman et al., 2010), or the hormonal status of the periods in adolescence development should be taken into consideration (Blakemore et al., 2010; Peper et al., 2011).

Considering phenotypes to map brain development it is important to realize that brain measures may capture different aspects of the brain, and that there are distinct genetic factors acting on these measures. Although measures like cortical volume, surface area, cortical thickness, sulcal depth, measures of cortical folding or gyrification are of course to some level associated with each other, they can capture more specific components of (cortical) development. This is not only important for the comparison between different groups of subjects, but also from a genetic perspective. Most of these measures have a genetic component (Kochunov et al., 2010; Rogers et al., 2010; Rogers et al., 2007), and therefore these measures could serve as an intermediate phenotype to facilitate the search for genetic factors or the actual genetic variants linked with healthy neurodevelopment or with psychopathologies. Furthermore, using or combining these measures holds promise for developmental studies (White et al., 2010), or for psychiatric disorders (White et al., 2003; Palaniyappan et al., 2011). Looking at individual differences in developmental brain changes is distinct from exploring individual differences at brain structures at a given age. As a result, this also has consequences for imaging studies exploring heritability of searching the genetic variants that could explain individual differences in developmental trajectories throughout puberty.

This thesis focuses on structural brain changes and cognition, and is one of the first to explore how changes in brain anatomy are linked to cognitive functions from a genetic perspective at two specific ages. A more integrative approach of structural and functional imaging will become more important in the future. Several studies have tried to link specific cognitive domains to brain regions

Table 9.1 Main findings of the studies described in this thesis.

Chapter	Aim	Main findings
3	To asses the effects of GA and BW on brain volumes and IQ in a population-based sample of children at the age of 9 (N=192 twins).	1) Shorter GA was associated with a relatively smaller CB volume at age 9. 2) Lower BW was associated with lower IQ scores at age 9.
4	Exploring the heritability of individual differences in dynamic measures of verbal learning ability in 9-year-old twins (N=112 pairs) and their older siblings (N=99)	1) Individual differences in verbal learning abilities were moderately heritable. The heritability of LS was 43% for both twins and siblings. Heritability for FS was 20% in 9-years-old twins and 30% in the older siblings.
5	To what extent contribute genetic and/or environmental factors to IQ scores at two age groups (9-11 years & 12-14 years) and on the stability of IQ scores across time.	1) Heritability increased for all IQ scales; FSIQ from 34% to 65%; VIQ from 37% to 51%; PIQ from 64% to 72%. Influences of C decreased over time; FSIQ from 43% to 18%; VIQ from 42% to 26%; PIQ, C was not significant. 2) Stability of FSIQ (r_p=.72) and VIQ (r_p=.72) was explained by influences of A and C. Stability of PIQ (r_p=.56) was completely explained by influences of A.
6	To explore to what extent brain volumes at age 9 (N=190) and 12 (N=125) are heritable, and whether volumetric brain changes are heritable. Furthermore, it was explored whether they are related to genetic factors influences the amount of overall increase in height.	1) Height and brain volumes were highly heritable at ages 9 / 12; height (93% / 93%); TB (93% / 96%); BB (93% / 96%); CB (95% / 95%); BB GM (88% / 92%); BB WM (88% / 88%); CB GM (93% / 80%); CB WM (64 % / 49 %); lateral ventricle (80% / 75%); third ventricle (61% / 59%). For all brain volumes high r_g over time were observed (>0.89). 2) All brain volumes increased, only BB GM volume decreased (-1.6%). Change in volume was heritable for TB (19%), BB (20%) en CB (45%). Change in BB volume was associated with change in CB (r_p =.49; r_g =.88; r_e =.34). 3) Change in height was heritable (73%) and partly correlated with changes in CB (r_p =.24; r_g =.48), but not BB.

7	To explore genetic influences on CT at age 9 (N=190) and 12 (N=125), and on the change in CT between ages 9 and 12. Furthermore, it was studied whether there are stable or age specific genetic factors acting on CT between the ages 9 and 12.	1)	CT was heritable at age 9 (mean CT=65%; local max. up to 78%), and at age 12 (mean CT=82%; local max. up to 91%)
		2)	Considerable thinning of the cerebral cortex was found between ages 9 and 12 (0.05 mm on average). This process was heritable (up to 79%), and the degree of genetic influence differs for the various areas of the brain.
		3)	Different genetic factors are responsible for variation in cortical thickness at ages 9 and 12, with independent genetic factors acting on cortical thickness across time and between various brain areas.
8	The association between brain structure and IQ was explored at two ages; childhood (age 9) and early adolescence (age 12). It was studied to what extent these associations are caused by shared genetic factors acting on both CT and IQ scores.	1)	At age 9, CT was not correlated with IQ scores, but at age 12 higher IQ scores were associated with a thinner cortex (r_p = -,32).
		2)	This effect was mainly driven by verbal IQ (mainly in left frontal cortex).
		3)	The correlations between IQ and CT were driven by shared genetic factors.

A = Additive genetic influences; BB = Cerebrum; BW = Birth Weight; C = shared environmental influences; CB = Cerebellum; CT = Cortical Thickness; GA = Gestational Age; FS = Forgetting Speed; GM = Gray Matter; IQ = Intelligence quotient; LS = Learning Speed; r_e / r_p / r_g = unique environmental / genetic / and phenotypic correlations; TB = Total Brain; WM = White Matter

or trying to locate IQ in the brain, but this is a challenging job (see for review, Cabeza and Nyberg, 2000; Deary et al., 2010). A more likely explanation is that not the anatomy or activation of a brain region is of importance, but that it is shaped by the activation within networks, involving multiple, interconnected regions (van den Heuvel and Hulshoff Pol, 2010). Cognitive maturation during adolescence is therefore not a direct reflection of brain regions that mature at different ages, but more of the development of networks obtaining more efficient strategies to perform a task (e.g., Casey et al., 2005a; Durston et al., 2006). Genetic influences on brain activation in neural networks supporting digit working memory tasks were found in an adult sample (Koten et al., 2009).

Many studies nowadays also explore the connectivity of the brain at rest, i.e. not performing a task during brain scanning (van den Heuvel et al., 2009; van den Heuvel and Hulshoff Pol, 2010). Genetic influences have been found for brain connectivity (Smit et al., 2008), activation of the default network (Glahn et al., 2010), and the cost-efficiency of functional network in adults (Fornito et al., 2011). A rapidly increasing number of studies now illustrate that indeed the development of the brain is not only reflected by structural changes, but is also associated with changes in connectivity between brain regions. Overall, long-range connections increase with age and short-range connections decrease with age. This indicates that the organizational characteristics of the brain network seem to differentiate from more local to a more distributed organization with increasing age (Fair et al., 2009; Supekar et al., 2009; Supekar et al., 2010; Dosenbach et al., 2010; Power et al., 2010).

9.4 Concluding remarks

This thesis aimed to gain insight how events around birth can have an effect on brain development and cognition in later life, to describe the extent to which genetic factors influence individual differences in brain development and cognition and to study the relation between brain structure and cognition at ages 9 and 12 years. In addition to studies exploring static brain structure and function during puberty, the dynamics of the adolescents brain will also become increasingly important in future research (Shaw et al., 2010; Karmiloff-Smith, 2010; Insel, 2010). To what extent genetics can explain individual differences in these developmental trajectories will become very important for the understanding of healthy but also disrupted brain development. Therefore, it is of great importance to embrace longitudinal projects, and continue ongoing projects. At this moment the children in the twin sample included in this thesis will return for a third follow-up. This will open up new opportunities for non-linear modelling of brain changes and cognitive functioning, and for exploring the link with functional MRI. Also the effects of more complex mechanisms of genes and environment are an important issue in future research. This will provide leads into fully understanding how individual differences in brain development occur to help every child to achieve it's best possible developmental trajectory.

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Nederlandse samenvatting

Genetische invloeden op structurele hersenontwikkeling en cognitie tijdens de jeugd

Inleiding

De puberteit is een belangrijke periode in de ontwikkeling van kindertijd naar volwassenheid. Niet alleen ondergaan de kinderen lichamelijke veranderingen (Sisk and Zehr, 2005), tijdens de puberteit is er ook een sterke vooruitgang in cognitieve vaardigheden zoals abstract beredeneren, plannen, het onderdrukken van impulsen of emotieverwerking (Forbes and Dahl, 2010). Hierbij speelt de ontwikkeling van de hersenstructuur en functie een belangrijke rol. Met behulp van magnetische spinresonantie beeldvorming (Magnetic Resonance Imaging; MRI), is het mogelijk om de structuur en functie van de hersenen te bestuderen. Bij deze beeldvormende techniek hoeven geen schadelijke stoffen of straling gebruikt te worden. Daarom is MRI onderzoek zeer geschikt voor herhaalde metingen in gezonde proefpersonen en dus ook kinderen. Uit eerder onderzoek is duidelijk geworden dat witte stof volume (bevat voornamelijk de uitlopers van neuronen die gezamenlijk verbindingen vormen) blijft toenemen van jonge leeftijd tot ver in volwassenheid (Giedd et al., 1999;Bartzokis et al., 2001). Grijze stof volume (bevat voornamelijk de cellichamen van neuronen) zal na een periode van groei in de kindertijd, rond de puberteit gaan afnemen in volume (Paus, 2005;Giedd et al., 2007). Deze afname van grijze stof is voornamelijk terug te zien in veranderingen in het volume van de hersenschors, maar ook in een verdunning hiervan (Sowell et al., 2002;Gogtay et al., 2004).

Aandoeningen, zoals ADHD ("Attention-Deficit/Hyperactivity-Disorder"), autisme en schizofrenie worden gezien als aandoeningen waarbij er iets mis gaat tijdens de ontwikkeling van de hersenen (Insel, 2010;Karmiloff-Smith, 2010;Shaw et al., 2010). Dit kan al zeer vroeg in het leven zijn, bijvoorbeeld voor de geboorte, of juist pas later optreden tijdens de puberteit. Het is bekend dat veel psychiatrische aandoeningen en de bijbehorende symptomen zich voor een eerste keer manifesteren tijdens of vlak na de puberteit. Veel van deze aandoeningen hebben ook een genetische achtergrond (Sullivan et al., 2003;Muhle et al., 2004;Faraone et al., 2005). Maar willen we iets kunnen zeggen over mogelijke invloeden op de ontwikkeling van hersenaandoeningen dan moeten we eerst de genetische of omgevingfactoren die ten grondslag liggen aan individuele verschillen in normatieve hersenontwikkeling en cognitie in kaart brengen.

Tweelingonderzoek is een belangrijk wetenschappelijk instrument binnen de geneeskunde en de psychologie. Dankzij onderzoek bij tweelingen kunnen we erachter komen in hoeverre verschillen in een bepaalde eigenschap worden beïnvloed door genen (erfelijke aanleg) of door leefomgeving. In het tweelingmodel worden de overeenkomsten op een bepaalde eigenschap binnen eeneiige (monozygote; MZ) vergeleken met de overeenkomsten binnen twee-eiige (dizygote; DZ) tweeling paren of tussen broers en zussen. Eeneiige tweelingen zijn genetisch identiek, terwijl twee-eiige tweelingen ongeveer de helft van hun erfelijk materiaal met elkaar delen. Als MZ tweelingen sterker op elkaar lijken op een bepaalde eigenschap in vergelijking met DZ tweelingen, zal deze eigenschap deels bepaald worden door genetische invloeden. Hoe groot de invloed van erfelijke factoren is, hangt af van het verschil in overeenkomst (vaak uitgedrukt in een correlatie) tussen MZ en DZ tweelingparen. Als MZ and DZ paren meer op elkaar lijken, dan op grond van genetica kan worden verklaard, is de gemeenschappelijke of "gedeelde" omgeving ook van belang. Kinderen die opgroeien in het zelfde gezin kunnen op elkaar lijken vanwege de omgeving die ze samen delen, dat blijkt bijvoorbeeld uit adoptieonderzoek. Tenslotte zijn er de "unieke" omgevingsfactoren die een deel van de variatie in eigenschappen verklaren. Dit zijn omgevingsfactoren die ervoor zorgen dat individuen, inclusief gezinsleden, van elkaar verschillen. De proportie van de totale variatie die toegeschreven kan worden aan de genetische variatie, wordt erfelijkheid ("heritability") genoemd (Falconer and Mackay, 1996;Boomsma et al., 2002). De decompositie van variatie binnen het klassieke tweelingmodel kan ook worden toegepast op de decompositie van covariatie tussen twee of meer eigenschappen of fenotypes. In dit

proefschrift wordt zowel de univariate als de multivariate methodes gebruikt om de oorzaken van variatie en covariatie te onderzoeken.

Er is veel onderzoek gedaan naar de erfelijkheid van cognitieve vaardigheden. De erfelijkheid van intelligentie neemt toe van kindertijd tot aan volwassenheid (Bartels et al., 2002;Haworth et al., 2010). De invloed van gedeelde omgevingsfactoren zijn van invloed in kindertijd, maar nemen af in de jonge adolescentie en verdwijnen uiteindelijk in volwassenheid (Bartels et al., 2002;Haworth et al., 2010). Individuele verschillen in hersenstructuur, o.a. hersenvolumes of dikte van hersenschors, is voor een zeer groot deel door genetische invloeden worden bepaald in volwassen, maar ook in kinderen (zie o.a. Peper et al., 2007;Schmitt et al., 2007). Er zijn ook studies die hebben laten zien dat erfelijkheid lijkt toe te nemen met leeftijd voor bijvoorbeeld grijze en witte stof volumes (Wallace et al., 2006), of voor de dikte van de cortex (Lenroot et al., 2009). Echter, dit zijn cross-sectionele studies en of dezelfde genetische factoren zijn op verschillende leeftijden hun effect hebben, of dat er specifieke genetische invloeden zijn voor bepaalde leeftijden, kan alleen beantwoord worden met longitudinale tweeling studies. Verder is het niet bekend in hoeverre genetische invloeden werken op structurele hersenveranderingen in deze periode en of dit samenhangt met cognitief functioneren.

Dit proefschrift

Het doel van dit proefschrift is om te onderzoeken in hoeverre genetische en omgevingsfactoren individuele verschillen tussen kinderen verklaren met betrekking tot de ontwikkeling van de hersenen en cognitieve vermogens. Voor dit onderzoek zijn data verzameld bij kinderen die ingeschreven staan bij het Nederlands Tweelingen Register (NTR) van de afdeling Biologische Psychologie van de Vrije Universiteit. In totaal hebben kinderen uit 112 gezinnen meegedaan bij de eerste meting. De tweelingen waren 9 jaar en in 103 families deed ook een oudere broer of zus mee. Van deze gezinnen zijn er 89 (bijna 80%) teruggekomen na 3 jaar, toen de tweelingen 12 jaar waren. Gegevens over onder meer zwangerschapsduur, geboortegewicht en mogelijke complicaties bij de geboorte waren beschikbaar uit vragenlijsten die waren ingevuld door de moeders vlak na de geboorte van de tweelingen. Gegevens over de cognitieve vermogens werden verzameld van de kinderen tijdens de bezoeken aan de Vrije Universiteit Amsterdam (VU) en het Universitair Medisch Centrum Utrecht (UMCU) met verschillende cognitieve testen, waaronder een psychometrische IQ test. Daarnaast werd de structuur van de hersenen bestudeerd op basis van herhaalde MRI scans die werden gemaakt tijdens de bezoeken aan het Universitair Medisch Centrum Utrecht. Een volledige beschrijving van de procedures en data verzameling staat beschreven in **hoofdstuk 2.**

Samenvatting van de studies in dit proefschrift

De studies beschreven in dit proefschrift onderzochten een breed scala van factoren die invloed kunnen hebben op individuele verschillen in hersenontwikkeling. Naast genetische en omgevingsinvloeden, waarvan het belang werd geschat met het klassieke tweelingonderzoek, werd gekeken naar de invloed van geboortegewicht en zwangerschapsduur. Er is onderzoek gedaan naar verschillende componenten van cognitie en hersenstructuur, naar hoe hersenstructuur en cognitie met elkaar geassocieerd zijn en de mate waarin een dergelijke associatie wordt verklaard door dezelfde genetische invloeden. Een overzicht van de belangrijkste bevindingen van elke studie opgenomen in dit proefschrift staat beschreven in Tabel 1.

Effecten van vroeggeboorte

Het doel van **hoofdstuk 3** was om te onderzoeken wat de gevolgen zijn van een kortere zwangerschapsduur en een lager geboortegewicht op hersenvolumes en cognitie op 9-jarige leeftijd. Veel eerder onderzoek is gericht op groepen kinderen met een verhoogd risico op hersenafwijkingen en verminderd cognitief functioneren op latere leeftijd in verband met zeer laag geboorte gewicht (< 1500 gram), of omdat ze zeer prematuur geboren zijn (< 32 weken). Het doel van mijn studie was om te onderzoeken wat de effecten zijn van zwangerschapsduur en geboortegewicht in een groep kinderen die niet per se behoren tot een hoog risico groep. De kinderen waren allemaal 9 jaar op het moment van de MRI scan en de bepaling van het intelligentie quotiënt (IQ) (N = 192 tweelingen) en waren geboren na een zwangerschapsduur tussen de 32 en 40 weken. Ze hadden een geboorte gewicht van 1525 tot 3820 gram. Uit de resultaten van deze studie bleek dat een kortere zwangerschapsduur was geassocieerd met een relatief kleiner cerebellum volume op 9-jarige leeftijd na corrigeren voor geboortegewicht, geslacht en leeftijd op het moment van de scan. Een lager geboortegewicht was ook geassocieerd met lager IQ op 9-jarige leeftijd, maar dit effect verdween wanneer er rekening werd gehouden met verschillen in intracraniële volume. Het cerebellum is een structuur welke vaker aangetast is bij kinderen die zeer vroeg of met een zeer laag geboorte gewicht ter wereld zijn gekomen (Allin et al., 2001; Allin et al., 2005). Het cerebellum is ook een structuur die juist in het laatste trimester van de zwangerschap nog relatief sterk groeit (Limperopoulos et al., 2005). Dit kan een verklaring zijn waarom specifiek zwangerschapsduur een effect heeft op cerebellum volume. De resultaten uit deze studie dragen bij aan het begrip dat lange termijn effecten van vroeggeboorte zich niet beperkt tot de groep zeer premature baby's of kinderen geboren met zeer laag geboorte gewicht.

Erfelijkheid van cognitie

In de studies beschreven in de **hoofdstukken 4** en **5,** is de invloed van genetische factoren op twee verschillende aspecten van cognitie onderzocht. In de studie beschreven in **hoofdstuk 4** werd de erfelijkheid onderzocht op individuele verschillen in verbaal geheugen. In deze studie deden 9 jarige tweelingen (N = 112 tweelingparen) en een oudere broer of zus tussen de 10 en 14 jaar (N = 99 individuen) mee. De kinderen kregen een lijst van 15 woorden te horen, die 5 keer achter elkaar werd afgespeeld. De kinderen werden gevraagd om zoveel mogelijk woorden te onthouden en op te noemen iedere keer nadat ze de reeks hadden beluisterd. vervolgens werden niet-lineaire leer curves werden bepaald voor elk kind, resulterend in twee parameters. De eerste parameter was leer snelheid ("Learning Speed"; LS) en representeerde het deel van het verbale materiaal wat nog niet herinnerd was in een vorige reeks, maar die wel werd herinnerd in een volgende reeks. De tweede was vergeet snelheid ("Forgetting Speed"; FS) en representeerde het deel van het verbale materiaal dat eerder met succes was onthouden en wat kan niet langer herinnerd werd in een volgende reeks. De belangrijkste conclusie van deze studie was dat de oudere kinderen in staat waren om meer nieuwe woorden toe te voegen aan hun werkgeheugen dan de jongere (aangegeven door een toename van LS). Individuele verschillen in verbaal leren waren matig erfelijk in de 9 jarigen (LS 43%; FS 20%), en in de 10 tot 14 jarigen (LS 43%; FS 30%).

Voor algemene cognitieve vaardigheden, zoals gemeten met een intelligentie test (Wechsler Intelligence Scale for Children –Third version; WISC-III), is de stabiliteit over leeftijd relatief hoog. In de studie beschreven in **hoofdstuk 5** bleek dat de longitudinale correlatie tussen de leeftijden van 9 tot 11 jaar en 12 tot 14 jaar was 0,72 voor totaal IQ, 0,72 voor verbaal IQ en 0,56 voor non-verbaal

IQ. Deze longitudinale IQ gegevens werden geanalyseerd in de tweelingen en hun broers en zussen om de vraag te beantwoorden in welke mate erfelijkheid kan verschillen voor totaal IQ, verbaal IQ en non-verbaal IQ tussen de twee leeftijdsgroepen. De voornaamste resultaten van dit hoofdstuk waren de toenames in erfelijkheid schattingen van de verschillende intelligentie schalen naarmate de kinderen ouder werden. Erfelijkheid van totaal IQ steeg van 34% in kindertijd tot 65% in de vroege adolescentie. De invloed van gedeelde omgevingsinvloeden nam af met de leeftijd voor totaal IQ, namelijk van 43% in de kindertijd tot 18% in de vroege adolescentie. Voor verbaal IQ werd een vergelijkbaar patroon van genetische en omgevingsinvloeden waargenomen. De erfelijkheid van verbaal IQ steeg van 37% in de kindertijd tot 51% in de adolescentie, terwijl de bijdrage van gedeelde omgevingsinvloeden daalde van 42% tot 26%. Voor non-verbaal IQ waren gedeelde omgevingsinvloeden niet van invloed. De erfelijkheid van non-verbaal IQ was 64% in de kindertijd en 72% in de vroege adolescentie. De stabiliteit van totaal en verbaal IQ werden verklaard door genetische en in mindere mate door gedeelde omgevingsinvloeden. De stabiliteit van non-verbaal IQ werd volledig bepaald door genetische invloeden. De resultaten van dit hoofdstuk bevestigen de bevindingen van toenemende erfelijkheid van algemene cognitieve capaciteiten tijdens de overgang van kindertijd naar adolescentie (Bartels et al., 2002;Hoekstra et al., 2007;Haworth et al., 2010). De verschillen in de genetische en omgevingsinvloeden op individuele verschillen in verbaal en non-verbaal IQ en in de stabiliteit van deze IQ schalen suggereren dat deze domeinen verschillende ontwikkeling trajecten hebben.

Erfelijkheid van structurele hersenveranderingen

De studies beschreven in **hoofdstuk 6** en **7** hadden als doel om in kaart te brengen in welke mate individuele verschillen in hersenstructuur worden verklaard door genetische invloeden. Dit werd onderzocht in tweelingen op de leeftijd van 9 (N = 190 kinderen) en 12 jaar (N = 125 kinderen). In **hoofdstuk 6** werd de erfelijkheid van verschillende hersenvolumes onderzocht op leeftijd 9 en 12. Daarnaast is onderzocht in hoeverre veranderingen in hersenvolumes tussen de leeftijden van 9 en 12 worden beïnvloed door genetische factoren. In **hoofdstuk 7** werd dit onderzocht voor de dikte van de cortex, en ook op de veranderingen in de dikte van cortex.

In **hoofdstuk 6** werden de volumes berekend van het totale brein, cerebrum, cerebellum, witte stof en grijze stof volume binnen het cerebrum en cerebellum en de laterale en derde ventrikels. Eerst werd er onderzocht wat de erfelijkheid was. Het bleek dat individuele verschillen in hersenvolumes sterk worden beïnvloed door genetische factoren op zowel 9- als 12-jarige leeftijd (erfelijkheid > 96%). Daarnaast was voor alle hersenvolumes de genetische correlatie (r_g) over tijd zeer hoog ($r_g > 0,89$). Een hoge genetische correlatie betekent dat dezelfde genetische factoren tot expressie komen op leeftijd 9 en leeftijd 12.

Tussen de leeftijd van 9 en 12 jaar, werd de groei van de hersenen bepaald. Totaal hersenvolume nam toe met 1,1% ten opzicht van het volume op 9-jarige leeftijd. De sterkste groei werd waargenomen in het cerebellum, wat een toename van 4,2% liet zien. Alleen cerebrum grijze stof volume nam af tussen 9 en 12 jaar (daling van 1,6%). Individuele verschillen in volume veranderingen bleek erfelijk te zijn, met een erfelijkheid van 20% voor verandering in cerebrum volume (totaal hersenvolume minus cerebellum en minus pons), en 45% voor verandering in cerebellum volume. De hoeveelheid groei in het cerebrum en de groei van het cerebellum waren geassocieerd met elkaar (fenotypische correlatie; $r_p = 0,49$), en dit verband werd gedreven door gedeelde genetische invloeden ($r_g = 0,88$). Daarnaast waren ook gedeelde unieke omgevingsinvloeden van belang ($r_e = 0,34$). Dus niet alleen is hersenvolume onder sterke invloed van genen maar ook hersenveranderingen zijn erfelijk.

De kinderen in deze leeftijd namen ook sterk toe in lichaamslengte en groeiden gemiddeld 9,9% in lichaamslengte tussen 9 en 12 jarige leeftijd. Individuele verschillen in lichaamsgroei waren erfelijk bepaald (73%). Lichaamsgroei bleek echter niet gecorreleerd met de toename in cerebrum volume. Veranderingen in lichaamslengte waren alleen voor een klein deel gecorreleerd met veranderingen in cerebellum volume (r_p = 0,24), wat deels werd verklaard door een overlap van genetische invloeden op veranderingen in lichaamslengte en cerebellum volume (r_g = 0,48). Naar aanleiding van deze studie kunnen we concluderen dat specifieke genetische processen invloed hebben op veranderingen van hersenvolumes, die niet kunnen worden verklaard door de toename in lichaamslengte tijdens dezelfde periode. Met andere woorden: wanneer kinderen hard groeien tussen de 9 en 12 jaar hoeft dat niet te betekenen dat hun hersenen in diezelfde periode ook hard groeien en vice versa.

De afname in grijze stof volume in het cerebrum was geassocieerd met verdunning van de cortex. In **hoofdstuk 7** werd onderzocht in hoeverre individuele verschillen in de dikte van de cortex worden verklaard door invloeden van genetische factoren op leeftijd 9 en 12. Verder werd ook onderzocht in hoeverre genetische factoren invloed hebben op de verdunning van de cortex. In dezelfde groep van 9 en 12 jarige tweelingen, zoals beschreven in **hoofdstuk 6,** werd de dikte van de cortex bepaald op meer dan 80.000 punten verdeeld over het gehele cerebrum. Individuele verschillen in corticale dikte bleken hoog erfelijk te zijn, zowel op 9 (maximaal erfelijkheid = 78%) als op 12 jarige leeftijd (maximale erfelijkheid = 97%). Tussen 9 en 12 jaar was duidelijk te zien dat er in meerdere gebieden corticale verdunning heeft plaats gevonden, met de sterkste veranderingen in de frontale, orbito-frontale, sensorische-motor en visuele gebieden. Individuele verschillen in dit proces van verdunning waren onder invloed van verschillende onafhankelijke genetische factoren (lokaal maximaal tot 79%). In sommige hersengebieden nam de invloed van deze genen over de jaren toe (in linkerhemisfeer taalgebieden Broca en Wernicke; in linkerhemisfeer zintuigelijke motor gebieden), terwijl in andere hersengebieden een uitwisseling van genetisch factoren leek plaats te vinden (in linker en rechter hersenhelft in de mediale frontale hersenschors). In deze gebieden was er een andere genetische factor op leeftijd op 9 jaar dan op 12 jaar. Dus, naast de hoge erfelijkheid van corticale dikte in kinderen op leeftijd 9 en 12, zijn genen van invloed op de verdunning van de cortex.

Het was al langer bekend dat gedurende de puberteit de structuur van de hersenen belangrijke veranderingen doormaakt, waaronder hersengroei, die samengaat met een opvallende verdunning van de hersenschors (Tiemeier et al., 2010; Gogtay et al., 2004; Sowell et al., 2002). De studies beschreven in dit proefschrift laten niet alleen zoals verwacht zien dat tussen de 9 en 12 jaar hersengroei samengaat met deze verdunning in de hersenschors, maar bovendien dat deze structurele veranderingen onder veranderende invloed van genen staat. Bij volwassenen was al bekend dat genen betrokken zijn bij veranderingen in de hersenschors (Brans et al., 2010). Deze studies beschreven in dit proefschrift laten zien dat dit ook het geval is bij kinderen.

Het verband tussen hersenstructuur en cognitie

In de studie beschreven in **hoofdstuk 8** werd onderzocht wat het verband was tussen corticale dikte en intelligentie (IQ). Verder werd onderzocht of dit verband door gedeelde genetische invloeden op IQ en corticale dikte verklaard werd. Van alle 9 en 12 jarige tweelingen waarvan MRI hersenscans gemaakt waren (zoals eerder beschreven in **hoofdstuk 6** en **7**) werd de cortex in 78 regio's verdeeld over het hele cerebrum. Van ieder gebied afzonderlijk werd vervolgens de gemiddelde corticale dikte berekend (ook wel "regions of interest" genoemd; ROI's). Totaal IQ score, verbaal en niet-verbaal IQ (eerder beschreven in **hoofdstuk 5**) werden gecorreleerd met de

corticale dikte op beide leeftijden. De belangrijkste bevindingen van deze studie waren dat op 9-jarige leeftijd de correlatie tussen corticale dikte en IQ beperkt en niet significant was. Op de leeftijd van 12 jaar ontstond er een correlatie tussen IQ en corticale dikte, waarbij een dunnere cortex geassocieerd was met een hoger IQ. Fenotypische correlaties bereikten een maximum van -0,32. De waargenomen correlatie tussen IQ en corticale dikte werd vooral gedreven door verbaal IQ en was voornamelijk gelokaliseerd in de linker frontale cortex (met correlaties van -0,21 tot -0,29). De correlaties tussen (verbaal) IQ en corticale dikte werden grotendeels verklaard door gedeelde genetische factoren tussen IQ en corticale dikte.

Eerdere studies rapporteren over het algemeen een positief verband tussen grijze stof volume en IQ. Daarom is het uiterst interessant om te weten hoe specifiek het effect van IQ is met corticale dikte. Om dit te kunnen vergelijken werden ook andere maten van corticale structuur onderzocht, zoals het totale oppervlakte van de cortex en corticale grijze stof volume. Beide maten om de structuur van de cortex te beschrijven waren positief gecorreleerd met niveau van intelligentie, terwijl corticale dikte een negatief verband had met intelligentie. Corticale dikte en grijze stof volume waren (genetisch) niet gecorreleerd met elkaar op de leeftijd van 9 (r_p = 0,09) en slechts matig gecorreleerd op leeftijd 12 (r_p = 0,24; r_g = 0,34). Corticale oppervlakte was relatief sterk gecorreleerd met corticale grijze stof volume (r_p = 0,72 op leeftijd 9; r_p = 0,69 op leeftijd 12).

Aan de hand van deze studie kunnen we concluderen dat hersengebieden die bij dragen aan verbale intellectuele prestaties zich lijken te specialiseren aan het begin van de puberteit onder de invloeden van gedeelde genen tussen verbale IQ en corticale dikte.

Ten slotte

Kinderen ondergaan aanzienlijke veranderingen in de hersenstructuur en functie gedurende de overgang van kindertijd naar puberteit. Er zijn niet alleen biologische veranderingen tijdens de periode van puberteit, maar ook emotionele veranderingen, die op hun beurt weer worden beïnvloed door veranderingen in gedrag. Daarom zal de mogelijke rol van omgeving (dat wil zeggen, de omgeving waaraan kinderen bloot worden gesteld, waarin ze opgroeien, of juist de omgeving die ze zelf opzoeken naarmate ze ouder worden), of de hormonale status (in welke puberteit fase) een belangrijke factor zijn in het onderzoek naar hersenontwikkeling in de puberteit. Aan de hand van tweelingstudies kan gekeken worden of individuele verschillen in een bepaalde eigenschap door genetische of omgevingsinvloeden verklaard worden. Het identificeren van de specifieke genen die hierbij betrokken zijn vereist echter studies met zeer grote aantallen aangezien steeds meer blijkt dat het om vele genen met elk een relatief klein effect op de eigenschap (zoals voor IQ; Deary et al., 2009).

Toekomst

Op dit moment zijn er verschillende initiatieven voor grote studies, veelal voortkomend uit een samenwerking tussen verschillende instituten over de hele wereld, om de genetische varianten te identificeren die samenhangen met IQ, hersenstructuur en hersenfunctie. Uit de onderzoeken beschreven in dit proefschrift is gebleken dat genetische effecten gekarakteriseerd worden door een hoge mate van stabiliteit over leeftijd (bijvoorbeeld IQ en brein volumes). Daarnaast zullen de ook de meer complexe wisselwerkingen tussen genen en de omgeving vormen een belangrijk onderwerp vormen van toekomstig onderzoek om volledig te begrijpen hoe individuele verschillen in de ontwikkeling van de hersenen optreden.

Tabel 1 Overzicht van alle hoofdbevindingen van de studies beschreven in dit proefschrift.

Hst	Doel	Hoofdbevindingen
3	Om te onderzoeken wat de gevolgen zijn van GA en BW op hersenvolumes en IQ scores op 9-jarige leeftijd (N=192 tweelingen).	1) Kortere zwangerschapsduur was geassocieerd met een relatief kleiner cerebellum volume op 9-jarige leeftijd. 2) Een lager geboortegewicht was geassocieerd met lager IQ op 9-jarige leeftijd.
4	Wat is de erfelijkheid voor verbaal geheugen in kinderen. Dit werd onderzocht in 9-jarige tweelingen (N=112 paren) en een oudere broer of zus (N=99 individuen).	1) Individuele verschillen in verbaal leren waren matig erfelijk. Erfelijkheid van LS was 43% in zowel de tweelingen als de oudere broers en zussen. Erfelijkheid voor FS was 20% in de tweelingen en 30% in de oudere broers of zussen.
5	Wat is de invloed van genetische en omgevingsfactoren op individuele verschillen in IQ scores in twee leeftijdsgroepen (kindertijd en adolescentie) en op de stabiliteit van IQ.	1) Erfelijkheid van totaal IQ steeg van 34% naar 65%; VIQ van 37% naar 51%; PIQ van 64% naar 72%. De invloed van C nam af met de leeftijd; totaal IQ van 43% naar 18%; VIQ van 42% naar 26%; PIQ (geen C 2) invloeden). Stabiliteit van totaal IQ (r_p= 0,72) en VIQ (r_p= 0,72) werden verklaard door A en C invloeden. De stabiliteit van PIQ (r_p= 0,56) werd volledig verklaard door invloeden van A.
6	Het bepalen van de erfelijkheid van hersenvolumes op leeftijd 9 (N=190) en 12 (N=125), en van volume veranderingen tussen 9 en 12 jaar. Daarnaast werd onderzocht of de invloeden van A op veranderingen in hersenvolumes gedeeld werden met de invloeden van A op lichaamsgroei.	1) Lichaamslengte en hersenvolumes waren zeer hoog erfelijk op leeftijd 9 en 12; Lengte (93% / 93%); TB (93% / 96%); BB (93% / 96%); CB (95% / 95%); BB GM (88% / 92%); BB WM (88% / 88%); CB GM (93% / 80%); CB WM (64 % / 49 %); laterale ventrikels (80% / 75%); derde ventrikel (61% / 59%). Voor alle hersenvolumes waren de r_g hoog over tussen 9 en 12 jaar (>0,89). 2) Alle hersenvolumes namen toe in volume, alleen BB GM nam af (-1,6%). Volume veranderingen waren erfelijk voor totaal hersenvolume (19%), BB (20%) en voor CB (45%). Groei in BB was geassocieerd met groei in CB (r_p = 0,49; r_g = 0,88; r_e = 0,34). 3) Lichaamsgroei was erfelijk (73%) en was deels gecorreleerd met volume veranderingen in CB (r_p = 0,24; r_g =0,48), maar niet met BB.

7	Het bepalen van de erfelijkheid van CT op leeftijd 9 (N=190) en 12 (N=125), en op CT veranderingen. Daarnaast werd onderzocht of er stabiele invloeden van A zijn over tijd of dat er invloeden van A op CT zijn voor specifieke leeftijden of gebieden over de cortex.	1) CT was erfelijk op 9 (gemiddeld CT=65%;lokaal tot aan 78%) en 12 jaar (gemiddeld CT=82%;lokaal tot aan 91%). 2) Substantiële verdunning van de cortex werd gevonden tussen de leeftijden 9 and 12 jaar (0.05 mm gemiddeld). 3) Corticale verdunning was erfelijk (max.=79%) en de mate van erfelijkheid verschilde per gebied. Verschillende genetische factoren waren verklaarde CT op leeftijd 9 en 12, met onafhankelijk genetische factoren over tijd en per gebied.
8	Om te onderzoeken wat het verband was tussen corticale dikte en intelligentie (IQ). Verder werd onderzocht of dit verband door gedeelde genetische invloeden op IQ en corticale dikte verklaard werd.	1) Op 9-jarige leeftijd geen correlatie tussen corticale dikte en IQ, maar op de leeftijd van 12 jaar was een dunnere cortex geassocieerd met een hoger IQ (max. r_p= -0,32). 2) De waargenomen correlatie tussen IQ en corticale dikte werd vooral gedreven door het verbale domein van IQ en was voornamelijk gelokaliseerd in de linker frontale cortex. 3) De correlaties tussen (verbaal) IQ en corticale dikte werden grotendeels verklaard door gedeelde genetische factoren.

A = Additieve genetische invloeden; BB = cerebrum; BW = geboortegewicht; C = Gedeelde omgevingsinvloeden; CB = cerebellum; CT = corticale dikte; GA = zwangerschapsduur; FS = Vergeet Snelheid; GM = Grijze stof; IQ = intelligentie quotient; LS = Leer Snelheid; r_e / r_p / r_g = unieke omgeving / genetische / en fenotypische correlaties; TB = Totaal brein; WM = Witte stof.

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Appendices

I Invitation letter parents (accompanied by Appendix II and III)
II Information brochure for parents
III Information brochure for children
IV Confirmation letter for parents
V Confirmation letter for children
VI Instruction brochure for urine and saliva collection
VII Instruction brochure for buccal swab collection
VIII Time schedule and checklist for visit UMCU
IX Informed consent for parents
X Informed consent for children

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlagen
Datum postmerk		020-598 8832	ouderfolder, kinderfolder
Ons kenmerk	**Uw kenmerk**	**Telefoon**	**E-mail**
NTR/IvS		020-598 8820 b.g.g 020-598 8792	ilc.van.soelen@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije* Universiteit *amsterdam

Geachte ouder/verzorger,

U ontvangt deze brief omdat u en uw kinderen ongeveer twee jaar geleden hebben meegedaan aan een groot onderzoek met als titel: ”**Tweelingonderzoek naar de ontwikkeling van brein en cognitie tijdens de pre-adolescentie**”. We zijn u nog steeds zeer dankbaar voor uw medewerking. Wij hebben u destijds verteld dat we de kinderen graag willen volgen in hun ontwikkeling, daarom ontvangt u nu deze uitnodiging. Net als toen werkt het tweelingenregister van de Vrije Universiteit (VU) nog steeds samen met het Universitair Medisch Centrum Utrecht (UMC Utrecht). Wij willen uw tweeling en eventueel hun broer of zus graag opnieuw vragen om mee te doen aan dit onderzoek.

Voor uw gemak willen wij zowel de verschillende testjes als de MRI-scan op een dag laten plaatsvinden. Dit zal deze keer allemaal gaan gebeuren in het UMC Utrecht. Aan het begin van de dag worden testen afgenomen, vergelijkbaar met die van de vorige keer en de dag zal worden afgesloten met het maken van een MRI-scan om onderzoek te doen naar de structuur van de hersenen.

Daarnaast zouden wij het zeer op prijs stellen als uw kinderen weer genetisch materiaal (verkregen met een monduitstrijkje), ochtendurine en speeksel zouden willen afstaan voor het onderzoek. Deze keer willen wij uw kinderen ook vragen om een vragenlijst in te vullen. Ook aan u willen we vragen om een monduitstrijkje af te staan en een soortgelijke vragenlijst in te vullen over uw kinderen. Tevens willen we u vragen een korte cognitieve test te doen die uw abstract redeneervermogen meet, ook als u dit de vorige keer al had gedaan. Door de gegevens te vergelijken met die van twee jaar terug, kunnen wij de betrouwbaarheid van onze gegevens verhogen. **Uiteraard wordt bij al het onderzoek de privacy van u en uw kinderen gewaarborgd.**

In bijgaande folders kunt u meer over het onderzoek lezen:
- Ouderfolder: deze folder bevat informatie over het onderzoek en de procedures
- Kinderfolder: deze folder bevat informatie over het onderzoek, speciaal voor kinderen

Z.O.Z. →

Ons onderzoek heeft als doel de normale ontwikkeling bij jonge gezonde kinderen in kaart te brengen. Het is niet de bedoeling om ziektes en/of afwijkingen op te sporen. Mocht er tijdens het onderzoek desondanks toch informatie naar boven komen die aanleiding geeft tot medisch handelen, dan wordt u daarvan op de hoogte gebracht. Wij zijn verplicht dit te doen; indien u dit niet wenst, kunt u niet meedoen aan het onderzoek. Het onderzoek zelf is onschadelijk voor de gezondheid. U en uw kinderen zijn uiteraard geheel vrij in uw keuze wat betreft deelname aan het onderzoek. Deelname aan het onderzoek kan op ieder moment door u of uw kinderen beëindigd worden.

Het onderzoek in het UMC Utrecht duurt ongeveer een dag, inclusief pauzes. De kinderen worden tijdens het eerste deel van de dag tegelijk getest, daarna worden ze na elkaar onderzocht in de MRI-scanner (dit duurt ongeveer 50 minuten per kind). We gaan vooraf een keertje oefenen in een oefenapparaat, zodat de kinderen weer vertrouwd kunnen raken met de procedure.
Wij zouden het prettig vinden als u op een doordeweekse dag kunt komen, maar zaterdagen zijn in sommige gevallen ook mogelijk. Voor wetenschappelijk onderzoek kan vrij gekregen worden van school als de school daar toestemming voor geeft. Uw reiskosten worden geheel vergoed en de kinderen krijgen na afloop een kleine attentie.

Binnenkort nemen wij contact met u op voor het maken van een afspraak en het beantwoorden van eventuele vragen. Mocht u na het lezen van de informatie direct al vragen hebben, dan kunt u contact opnemen met Inge van Soelen (VU, Amsterdam) tel: 020-598 8820; zij zal deze keer het onderzoek coördineren.

Het is ook mogelijk dat u liever een deskundige wilt spreken die niet direct betrokken is bij dit onderzoek om een onafhankelijk advies te krijgen. Dhr. Prof. dr. Ph. Scheltens, arts (VU medisch centrum, Amsterdam), is bereid u daartoe te woord te staan. U kunt via zijn secretaresse een afspraak maken, tel: 020-444 3222.

Wij hopen u en uw kinderen weer bij dit onderzoek te mogen verwelkomen!

Met vriendelijke groet,

Prof. dr. D.I. Boomsma

Mede namens:
prof. dr. R.S. Kahn
dr. H.E. Hulshoff Pol
drs. Inge van Soelen

Bijlagen:

- Ouderfolder
- Kinderfolder

INFORMATIEFOLDER OUDERS

Tweelingonderzoek naar de ontwikkeling van brein en cognitie tijdens de pre-adolescentie

Tweelingonderzoek naar de ontwikkeling van brein en cognitie tijdens de pre-adolescentie

vrije Universiteit amsterdam

UMC Utrecht

ALGEMENE INFORMATIE OVER TWEELINGONDERZOEK

Tweelingonderzoek is een belangrijk wetenschappelijk instrument binnen de geneeskunde en de psychologie. Dankzij onderzoek bij tweelingen kunnen we erachter komen in hoeverre verschillen in gedrag en het krijgen van bepaalde ziektes worden beïnvloed door genen (erfelijke aanleg) of door leefomgeving. Om goed onderzoek te kunnen doen is het nodig om gegevens te verzamelen van grote groepen familieleden zoals tweelingen, ouders en kinderen of broers en zussen. Bovendien is het belangrijk om onderzoek niet eenmalig te doen, maar om tweelingen en andere familieleden te volgen tijdens hun ontwikkeling. Daarom is in 1987 aan de Vrije Universiteit (VU) te Amsterdam het Nederlands Tweelingen Register (NTR) opgericht. Bij het NTR staan momenteel 30.000 tweelingparen ingeschreven. De meeste gegevens over deze tweelingen zijn verkregen met gedragsvragenlijsten die door de tweelingen zelf of door hun ouders en leerkrachten zijn ingevuld. Verschillende wetenschappers maken gebruik van deze gegevens om zo beter te begrijpen welke invloed genen en omgeving hebben op groei en ontwikkeling, persoonlijkheid, gedrag en emotionele problemen (bijvoorbeeld ADHD) van kinderen, gezondheid en leefgewoonten. De belangeloze medewerking van tweelingen en hun families is daarbij van onschatbare waarde! Soms worden groepen tweelingen en hun familieleden ook uitgenodigd om mee te doen aan onderzoek dat niet met een vragenlijst alleen gedaan kan worden. Het onderzoek waar u 2,5 jaar geleden aan meedeed is daar een voorbeeld van. Nu nodigen we u opnieuw uit.

INFORMATIE OVER HET HUIDIGE ONDERZOEK

Voor dit onderzoek werken het NTR en het Universitair Medisch Centrum Utrecht (UMCU) samen. U hebt met uw kinderen in het verleden al meegedaan aan de eerste meting binnen dit onderzoek.
We zijn u en uw kinderen nog steeds dankbaar voor uw deelname. Nu willen wij u graag nog een keer uitnodigen voor de vervolgmeting binnen dit onderzoek. In het kader van dit onderzoek wordt gekeken naar de genetische achtergrond van hersenontwikkeling, geheugen, leesvaardigheid en leervermogen. Hierdoor hopen we in de eerste plaats meer te weten te komen over hoe het komt dat mensen verschillen in hun verstandelijke vermogens, wordt dit vooral veroorzaakt door verschillen in de genen of vooral door verschillen in de omgeving? Tevens willen we meer te weten komen over de invloed van geslachtshormonen op de ontwikkeling van de hersenen, het verstandelijk vermogen en over de samenhang tussen ontwikkeling van de hersenen en het vermogen om te leren. We hopen met dit onderzoek meer inzicht te krijgen in de normale ontwikkeling van de hersenen. Alleen als dezelfde deelnemers meerdere keren meedoen aan onderzoek kan in kaart worden gebracht hoe kinderen zich ontwikkelen in de vroege puberteit.
Wij willen in dit onderzoek zowel uw tweeling als een broertje of zusje van de tweeling nogmaals uitnodigen. Wij willen graag eeneiige, tweeeiige tweelingen en hun broertjes en zusjes onderzoeken, omdat door deze combinatie het beste na te gaan is welke eigenschappen erfelijk zijn, welke eigenschappen vooral door gedeelde omgevingsfactoren worden veroorzaakt (in hetzelfde gezin opgegroeid) en welke eigenschappen bepaald worden door factoren die voor ieder kind en mens uniek zijn. Dit kunnen we onderzoeken aangezien eeneiige tweelingen uit één gezin dezelfde genen (erfelijk materiaal) hebben en in dezelfde omgeving zijn opgegroeid. Verschillen tussen helften van een eeneiige tweeling worden dus veroorzaakt door verschillen in de unieke omgeving. Van de broertjes en zusjes van de tweeling weten we dat ze in dezelfde omgeving zijn opgegroeid als de tweeling, maar dat gemiddeld slechts de helft van hun genen gelijk zijn aan die van de andere broers en zussen. Ditzelfde geldt ook voor twee-eiige tweelingen. Door nu zowel de gegevens van eeneiige en twee-eiige tweelingen en hun broertjes en zusjes met elkaar te vergelijken, kunnen we bepalen hoe groot de invloed van genen, gedeelde omgeving en unieke omgeving op de ontwikkeling van hersenen en leervermogen is.
Ongeveer 2,5 jaar geleden deden veel 9-jarige tweelingen, hun ouders en broers en zussen mee. Nu willen wij deze groep opnieuw uitnodigen om de ontwikkeling van de hersenen en de verstandelijke vermogens te kunnen onderzoeken. Tussen 9 en 11 jaar laten de meeste kinderen de eerste tekenen van de puberteit zien. Het is daarom een belangrijke periode in de ontwikkeling van kinderen.
Het onderzoek zal één dag duren en zal plaats vinden in het UMCU (u hoeft dus niet ook nog naar de VU in Amsterdam te komen). In de ochtend zullen er testen afgenomen worden om geheugenfunctie, intelligentie en het leervermogen te bepalen. Deze testen zijn voor een groot

deel gelijk aan de testen van 2,5 jaar geleden. In de middag wordt een hersenscan gemaakt. Naast de testresultaten en de hersenscan is het belangrijk goede informatie te hebben over hoever de kinderen in de puberteit zijn. Daarom zal er een kort lichamelijk onderzoek plaatsvinden en worden hormoonspiegels bepaald. Tenslotte willen we opnieuw DNA verzamelen. Dit is ten bate voor toekomstig onderzoek naar specifieke genen die groei, ontwikkeling van de hersenen, cognitieve vaardigheden of leervermogen beïnvloeden. Materiaal voor hormoonspiegels en DNA bepalingen kunnen thuis worden verzameld en worden meegebracht naar de afspraak op het UMCU. Meer gedetailleerde informatie over de verschillende procedures vindt u in de onderstaande teksten.

INTELLIGENTIE

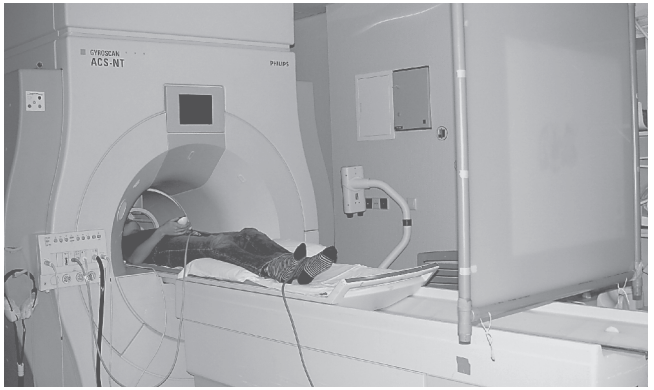
Iemands intelligentie (mentale vaardigheid) kunnen we meten met een IQ-test. Deze test bestaat uit een groot aantal vragen, waar vaak zo snel mogelijk het juiste antwoord op moet worden gegeven. Sommige vragen testen het taalbegrip, andere testen ruimtelijk inzicht. De totaalscore op de verschillende onderdelen van de test resulteert in een bepaald getal, het IQ. In dit onderzoek worden de WISC-III en de Raven test (deze test meet vooral abstract redeneren) afgenomen. Aan de ouders wordt ook gevraagd de Raven in te vullen, om te onderzoeken of er een relatie is tussen de scores van ouders en kinderen en tussen de ouders onderling. Ook als de Raven in het verleden al een keer is ingevuld, willen we graag vragen of deze ouders het nog een keer willen invullen. Deze herhaling is voor ons belangrijk, omdat we dan de betrouwbaarheid van deze test kunnen onderzoeken over een grotere tijdsperiode (namelijk ongeveer 2,5 jaar).

LEERVERMOGEN

We meten het leervermogen met behulp van een aantal taken. Deze taken meten verschillende aspecten van leren zoals, aandacht, verbaal vermogen, korte en lange termijn geheugen en herinneren van bepaalde patronen. Zo zal er onderzocht worden hoe goed de kinderen een lijstje woorden kunnen onthouden, ook wordt er een korte leestest afgenomen om te kijken of er mogelijk sprake is van leesproblemen.
Een gedeelte van de taken zal op een computer afgenomen worden. Het andere deel zal door de onderzoekers afgenomen worden. Veel kinderen vinden het leuk om de taakjes zo snel en nauwkeurig mogelijk te volbrengen.

HERSENSCAN

De hersenscan zal gemaakt worden met behulp van een MRI-apparaat. De MRI-techniek is gebaseerd op radiogolven en magnetische velden en is daardoor niet schadelijk voor de gezondheid. Voordat er een MRI-scan wordt gemaakt van de hersenen, krijgen de kinderen een uitleg over de scanprocedure en kunnen ze nog een keertje alle handelingen oefenen in de speciaal daarvoor bestemde oefenscanner (zie foto). Vervolgens kunnen ze de eventuele metalen voorwerpen die zij nog bij zich dragen, afdoen in de hiervoor bestemde kleedruimte (persoonlijke eigendommen kunnen veilig worden opgeborgen). Om er zeker van te zijn dat de



MRI-scanner

kinderen geen metalen voorwerpen meer bij zich dragen op het moment dat zij de scanruimte ingaan, worden hierover door de onderzoeker nog enkele gerichte vragen gesteld. Hierna kan het scannen van de hersenen beginnen.

Tijdens het MRI-onderzoek liggen de kinderen op een bed. Dit bed wordt door een laborant gedeeltelijk een tunnel ingeschoven. Deze heeft een diameter van 60 cm en is aan beide kanten open. Het duurt ongeveer 50 min. om de foto's te maken. Gedurende de scan mag uw kind zich niet bewegen en maakt het apparaat harde geluiden. Tijdens de scan kan naar een film op DVD gekeken worden of naar zelf meegebrachte muziek op CD worden geluisterd. Op ieder moment kunnen de kinderen aangeven te willen stoppen met het onderzoek.

VRAGENLIJST

Omdat we ook graag meer willen weten over het gedrag van de kinderen, hebben we zowel voor de kinderen als voor de ouders vragenlijsten samengesteld. De vragenlijst voor de ouders kan zelfstandig thuis worden ingevuld en meegenomen worden naar de testdag in Utrecht. De vragenlijst voor de kinderen bestaat uit twee delen. Het eerste deel kan thuis door het kind zelfstandig worden ingevuld en meegenomen worden naar het UMCU. Het tweede deel van deze vragenlijst beslaat een paar korte vragen en kan ingevuld worden op de testdag zelf bij de testleider.

PUBERTEIT

Om vast te stellen hoever de kinderen in de puberteit zijn, wordt er op het UMCU een onderzoek uitgevoerd waarbij de lichamelijke ontwikkeling van de kinderen wordt vastgesteld. De kinderen moeten zich daarvoor uitkleden. Daarnaast vragen we de kinderen thuis ochtendurine en speeksel te verzamelen om de hoeveelheid geslachtshormonen te kunnen bepalen.

CORTISOL

Cortisol is een hormoon dat vooral bekend staat als stresshormoon. Het is ook van invloed op de werking van de hersenen en kan gemeten worden in speeksel. Om de hoeveelheid cortisol te bepalen, vragen we de kinderen twee dagen gedurende de ochtend een aantal keer op een wattenrolletje te kauwen.

DNA

Door middel van het vergelijken van tweelingen met elkaar en met hun broertjes en/of zusjes kan worden nagegaan hoe groot erfelijke en omgevingsinvloeden zijn op bepaalde eigenschappen. Het is voor dit gedeelte van het onderzoek nog niet nodig om het erfelijke materiaal zelf, namelijk het DNA, te bestuderen. Als we echter willen weten welke genen verantwoordelijk zijn voor verschillen in ontwikkeling van de hersenen en leervermogen, moeten we het DNA bestuderen.
Met een wanguitstrijkje kan op een eenvoudige en pijnloze manier DNA verzameld worden. Dit wordt gemaakt door met een wattenstaafje zachtjes langs de binnenkant van de mond te wrijven. (De cellen van het wangslijmvlies worden zeer vaak vernieuwd, daarom zijn deze cellen bij uitstek geschikt voor verzameling van DNA.) Dit kan gewoon thuis worden gedaan. De wattenstaafjes kunnen worden meegenomen naar het UMCU.

PRIVACY

Alle gegevens zijn strikt vertrouwelijk en vallen onder de strenge privacy-regels van de VU en het UMCU. Alle procedures en onderzoeksmethoden uit deze studie zijn getoetst door de Centrale Commissie Mensgebonden Onderzoek (CCMO).
De gegevens die met dit onderzoek worden verzameld, worden altijd 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. Tevens zullen de gegevens de VU en het UMCU niet verlaten. De gegevens kunnen wel worden ingezien door medewerkers die bij het onderzoek betrokken zijn, door de Gezondheidsinspectie en door de Medisch-Ethische Toetsingscommissie.

VERZEKERING

Deelname aan het onderzoek valt onder de aansprakelijkheidsverzekeringen van de VU en het UMCU. De verzekering van het VU medisch centrum loopt bij Onderlinge Waarborg maatschappij Centramed b.a. Bij letselschade kunt u contact opnemen met Dhr. Pardoen op telefoonnummer 070-3762185. Deze verzekering vergoed € 450.000,- met een maximum van € 3.500.000,- voor het hele onderzoek en een maximum van € 5.000.000,- voor alle onderzoeken verricht bij de VU. De verzekering van het UMCU loopt bij het Marketform Limited te London. De tussenpersoon van deze verzekering is de heer H. Melinga. Deze is te bereiken bij CenE verzekeringen b.v. op telefoonnummer 030-2560406. Deze verzekering vergoedt letselschade die zou kunnen ontstaan door deelname aan onderzoek. De verzekering biedt dekking voor schade tot een maximum bedrag van € 500.000 per deelnemer en € 6.900.000 voor de schade van alle proefpersonen tezamen die deelnemen aan dit onderzoek. Voor de totale schade die zich per verzekeringsjaar bij proefpersonen heeft geopenbaard bij alle onderzoeken die de opdrachtgever verricht, is het verzekerd bedrag gelimiteerd tot € 9.100.000 per verzekeringsjaar. Voor beide verzekeringen geldt dat uitgesloten van dekking is schade:

a waarvan nagenoeg zeker was dat deze zich bij de proefpersoon zou voordoen;
b die zich bij nakomelingen openbaart als gevolg van een nadelige inwerking van het onderzoek op het genetisch materiaal van de proefpersoon;
c door aantasting van de gezondheid van de proefpersoon die zich ook zou hebben geopenbaard wanneer de proefpersoon niet aan dit onderzoek had deelgenomen;
d die het gevolg is van het niet, niet volledig of foutief opvolgen van de aanwijzingen en instructies die u door de onderzoeker zijn gegeven en welke in de proefpersooninformatiebrief zijn verwoord.

PRAKTISCHE INFORMATIE

✔ U bent geheel vrij in het al dan niet deelnemen aan ieder deel van het onderzoek.

✔ Er kan altijd zonder opgaaf van redenen worden gestopt met deelname aan het onderzoek.

✔ De onderzoekers handelen volgens de gedragscode verzet van de Nederlandse Vereniging van Kindergeneeskunde. Als blijkt dat een van uw kinderen gedurende het onderzoek niet verder wil gaan met het onderzoek, zal het onderzoek met dit kind direct worden afgebroken.

✔ Gegevens die uit het cognitieve onderzoek naar voren komen en die voor eventuele behandeling van belang zijn, worden besproken. Mochten er bijkomende bevindingen zijn naar aanleiding van het MRI-onderzoek, waarvoor medisch handelen noodzakelijk wordt geacht, dan wordt de betrokkene (of na toestemming eventueel ook de huisarts) hiervan op de hoogte gesteld.

✔ Als u een deskundige wilt spreken die niet direct betrokken is bij dit onderzoek om een onafhankelijk advies te krijgen, kunt u terecht bij Dhr. Prof. dr. Ph. Scheltens, arts (VU medisch centrum). U kunt via zijn secretaresse een afspraak maken, tel. 020-4443222.

✔ De reiskosten die gemaakt zijn, worden vergoed.

✔ Het is de bedoeling dat de tweeling (plus eventuele broertjes en zusjes) samen op dezelfde dag langskomen om te worden getest of gescand.

✔ Voor eten en drinken wordt gezorgd.

✔ Kinderen krijgen na afloop van het onderzoek een tegoedbon om zelf een cadeautje uit te zoeken bij een speelgoedwinkel of een platenzaak

✔ Indien u het op prijs stelt worden de belangrijkste resultaten van het wetenschappelijk onderzoek thuisgestuurd. Ditzelfde geldt voor de uitslagen van de testen en een plaatje van de hersenen.

✔ In de toekomst kunt u nogmaals benaderd worden voor vervolgonderzoek, tenzij u aangeeft hier geen prijs op te stellen.

INFORMATIEFOLDER KINDEREN

Tweelingonderzoek naar de ontwikkeling van leren, geheugen en hersenen

Tweelingonderzoek naar de ontwikkeling van leren, geheugen & hersenen

vrije Universiteit amsterdam

UMC Utrecht

TWEELINGONDERZOEK

Bij de Vrije Universiteit Amsterdam en de Universiteit van Utrecht zijn we bezig met een groot onderzoek bij tweelingen en hun broers en zusjes. Ongeveer 2,5 jaar geleden hebben jullie ook al meegedaan. Nu willen we jullie graag nog een keer uitnodigen, zodat we kunnen gaan kijken hoeveel jullie zijn veranderd. In deze folder kun je lezen waar dit tweede gedeelte van het onderzoek precies over gaat en waarom we jullie graag nog een keertje willen onderzoeken.

HET ONDERZOEK

We willen graag weten waarom sommige kinderen beter kunnen leren dan andere kinderen. We willen onderzoeken of dit te maken heeft met de bouw en werking van de hersenen.

Verder willen we graag weten hoe kinderen veranderen als ze ouder worden. We zouden het heel fijn vinden als je nog een keer mee wilt doen met dit onderzoek, zodat we kunnen zien welke veranderingen er zijn opgetreden.

Daarom willen we dezelfde testjes als 2,5 jaar geleden nog een keer doen. Bovendien gaan we opnieuw foto's maken van je hersenen. Zo kunnen we onderzoeken hoe de hersenen veranderen als je opgroeit. Als je wilt, kun je net als de vorige keer de foto's van je hersenen krijgen om misschien aan anderen te laten zien of boven je bed te hangen.

Voor dit onderzoek kom je een dag naar het Medisch Centrum in Utrecht. In de ochtend krijg je een aantal testjes en in de middag maken we de foto's van je hersenen.

WAAROM TWEELINGEN?

Je lichaam bestaat uit heel veel kleine cellen waarin overal een kern zit met DNA. Hoe je eruitziet wordt grotendeels bepaald door je DNA. Je DNA zorgt er bijvoorbeeld voor dat je blauwe of bruine ogen hebt. Dit DNA komt van je ouders, je hebt de ene helft van je moeder en de andere helft van je vader gekregen. Soms lijken broers en zussen heel erg op elkaar, dat komt omdat ze dan voor een gedeelte hetzelfde DNA van hun ouders hebben gekregen. Maar ze krijgen niet altijd precies dezelfde stukjes als hun broers of zussen. Daarom kan het zijn dat ze toch wat verschillen van elkaar. Twee broers kunnen bijvoorbeeld wel dezelfde kleur haren hebben, maar een andere kleur ogen.

Het bijzondere van eeneiige tweelingen is dat ze precies hetzelfde DNA hebben. Daarom lijken zij wel zoveel op elkaar. Twee-eiige tweelingen hebben niet precies hetzelfde DNA en ze lijken daarom net zoveel op elkaar als gewone broers en zusjes. Maar hoe zit het dan met dingen waar je wel of niet goed in bent? Sommige kinderen zijn heel goed in hoofdrekenen en andere kinderen zijn heel goed in sport. Heeft dat ook te maken met je DNA?

Dat hoeft niet altijd zo te zijn. Soms verschillen kinderen van elkaar omdat ze andere dingen meemaken in hun omgeving. Kinderen kunnen bijvoorbeeld een andere schooljuf hebben, of sommige kinderen zitten bij een voetbalclub terwijl een ander kind liever een boek leest.

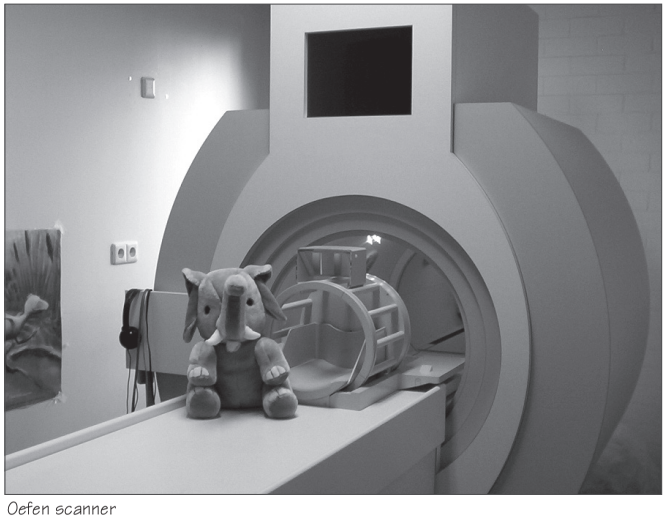
Wij willen graag weten of kinderen van elkaar verschillen omdat ze ander DNA hebben (is het erfelijk?) of omdat ze andere dingen meemaken. Om dit uit te zoeken hebben we tweelingen en hun broers en zussen nodig. Als eeneiige tweelingen meer op elkaar lijken dan twee-eiige tweelingen en op hun broers en zusjes, dan heeft dat waarschijnlijk te maken met het DNA dat bij hen hetzelfde is. Als er geen verschillen zijn in hoeveel eeneiige en twee-eiige tweelingen en broertjes en zusjes op elkaar lijken, dan weten we dat erfelijkheid niet belangrijk is. Verder willen we ook graag weten of tweelingen verschillen van hun broertjes en zusjes. We zouden dus heel blij zijn als jullie nog een keer mee zouden doen aan dit onderzoek.

TESTJES

We beginnen de dag met ongeveer dezelfde testjes als de vorige keer, waarmee we onderzoeken hoe goed je kunt leren en onthouden nu je ouder bent geworden. Misschien kan je ze nog wel herinneren. Sommige testjes bestaan uit vragen, terwijl bij andere het erom gaat om zo snel mogelijk te reageren. Soms moet je een soort puzzel maken. Een aantal van deze testjes staat op een computer, maar soms worden de vragen ook door de onderzoeker gesteld. De meeste kinderen vonden de testjes vorige keer leuk om te doen en vonden het ook leuk om zo snel mogelijk te reageren. Dus we hopen dat jullie het nu ook weer leuk zullen vinden. Het testen

duurt ongeveer een paar uur met een aantal pauzes. Voor eten en drinken wordt gezorgd. Als je wilt, sturen we je later een overzicht, net als de vorige keer zodat je kan zien hoe je de testjes hebt gedaan.

FOTO'S VAN JE HERSENEN



Oefen scanner

In de middag worden opnieuw foto's van je hersenen gemaakt in een MRI-apparaat. Dit wordt ook wel een scanner genoemd. Het zal net als de vorige keer gaan.
Je komt in een soort tunnel te liggen en je zult niets voelen wanneer de foto's worden gemaakt. Het apparaat maakt nog wel wat lawaai. Het kan zijn dat je het toch nog een beetje eng vindt. Dat is helemaal niet erg, want we gaan eerst nog een keer oefenen in de 'oefen scanner' (zie plaatje), voordat we de echte foto's gaan maken. Tijdens het maken van de foto's mag je weer naar een DVD kijken of naar muziek luisteren die je zelf hebt meegenomen. Het zal ongeveer 50 minuten duren om alle foto's te maken. Je mag op ieder moment aangeven dat je wilt stoppen met het onderzoek. Dit geldt voor alle onderdelen van het onderzoek. Je hoeft niet te zeggen waarom je wilt stoppen.
Wil je meer weten over een MRI scanner? Kijk dan eens op onze website http://www.brainsforkids.nl/Home. Wil je alvast een keertje oefenen voor in de MRI scanner, kijk dan eens op www.niche-lab.nl/oefenscan.html.

VRAGENLIJSTEN

Dit keer hebben we twee boekjes samengesteld met verschillende vragen. Het eerste boekje krijg je thuisgestuurd en mag je thuis invullen en meenemen als je naar Utrecht komt. Ook je ouders krijgen van ons een boekje met vragen om thuis in te vullen. In Utrecht krijg je het tweede boekje, wat je op de testdag zelf mag gaan invullen. Aan het einde van de dag kan je het ingevulde boekje weer terug geven aan de onderzoeker.

VOLWASSEN WORDEN

Als je volwassen wordt, gaat je lichaam zich verder ontwikkelen en dat wordt ook wel de puberteit genoemd. De puberteit begint als in je lichaam meer speciale hormonen gaat aanmaken. Deze speciale hormonen zijn stoffen die tegen het lichaam zeggen dat het volwassen moet worden.

Hierdoor krijgen jongens en meisjes bijvoorbeeld allebei haren onder de armen en rond de geslachtsdelen. Deze hormonen zitten ook in je speeksel en je urine. Om te kunnen meten hoeveel hormonen er nu al aanwezig zijn, vragen we of je nog een keer wat ochtendplas en speeksel wilt verzamelen. Daarnaast kunnen we ook aan je lichaam zien of je al in de puberteit bent. Bij het bezoek in Utrecht zal daarom een kort lichamelijk onderzoek worden gedaan door de onderzoeker waarvoor je je even moet uitkleden. Het is belangrijk om te weten hoe ver je al in de puberteit bent, omdat dan ook je hersenen gaan veranderen.

DNA

Aan de binnenkant van je wang zitten losse cellen. Als je zachtjes met een wattenstaafje langs de binnenkant van je wang gaat, blijven die cellen op het wattenstaafje achter. Uit deze cellen kunnen wij DNA halen. Vorige keer hebben jullie dit al een keer voor ons gedaan om te kijken of jullie eeneiig of twee-eiig waren. Voor dit onderzoek vragen wij jullie om nog een keer met een wattenstaafje langs je wang te schrapen en dit mee te nemen naar Utrecht. Wij gebruiken dit dan voor in de toekomst als we op zoek gaan naar welke stukjes DNA nu kunnen bepalen hoe je hersenen eruit zien, of waarom de een beter is in rekenen terwijl een ander liever wil tekenen.

TENSLOTTE

Als dank voor je medewerking krijgen jullie aan het eind van het onderzoek een tegoedbon, waarmee jullie zelf een cadeautje kunnen uitzoeken. Als jullie dat leuk vinden, krijgen jullie ook de uitslagen van de testen en een foto van jullie hersenen opgestuurd.

MEER INFORMATIE

Als jullie nog vragen hebben, kunnen jullie in de folder van jullie ouders kijken, of bellen of mailen naar de onderzoeker. De onderzoeker die de testjes doet en de hersenfoto's maakt in Utrecht, heet Inge van Soelen.

Inge van Soelen:
020-5988820 / email: ilc.van.soelen@psy.vu.nl

Als je liever je vragen wilt stellen aan iemand die niets met dit onderzoek te maken heeft, maar er wel veel van af weet, kun je je vragen stellen aan dokter **Scheltens.** Je kunt hem bereiken door te bellen met zijn secretaresse op telefoonnummer 020-4443222.

Als je meer wilt weten over tweelingenonderzoek in het algemeen, kun je een kijkje nemen op de website van het Nederlands Tweelingen Register: www.tweelingenregister.org

Als je meer wilt weten over een MRI scanner of alvast een keertje wilt oefenen, kijk dan op de speciaal voor kinderen gemaakte websites: http://www.brainsforkids.nl/Home of www.niche-lab.nl/oefenscan.html

Inge van Soelen
telefoon: 020-5898820
e-mail: ilc.van.soelen@psy.vu.nl

Vrije Universiteit Amsterdam
Biologische Psychologie
Van der Boechorststraat 1
1081 BT Amsterdam

Universitair Medisch Centrum Utrecht
Afdeling Psychiatrie
Heidelberglaan 100
3584 CX Utrecht

Nederlands Tweelingen Register (NTR)

Datum	Uw brief van	Telefax	Bijlage(n)
datum postmerk		020-598 8832	diversen
Ons kenmerk	**Uw kenmerk**	**Telefoon**	**E-mail**
NTR/IvS		020-598 8820 b.g.g 020-598 8792	ilc.van.soelen@psy.vu.nl

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije* Universiteit *amsterdam

Geachte ouder en/of verzorger,

Allereerst willen wij u hartelijk bedanken voor uw medewerking aan ons onderzoek. Zoals u al heeft kunnen lezen in de vorige brief, ontvangen u en uw kinderen de aanwijzingen voor het verzamelen van het lichaamsmateriaal. In deze envelop treft u de gebruiksaanwijzing en de buisjes voor het verzamelen van monduitstrijkjes aan.

Uw kinderen ontvangen ieder twee eigen enveloppen die, naast de buisjes en de gebruiksaanwijzing voor het verzamelen van DNA, ook gebruiksaanwijzingen en buisjes voor het verzamelen van speeksel en ochtendurine bevat. Wilt u zo vriendelijk zijn om ze hierbij te helpen?

Het is de bedoeling dat u en uw kinderen het lichaamsmateriaal verzamelen in de week voorafgaand aan uw afspraak op het UMC Utrecht, **«Afspraak_UMC»**. Ook is er voor de kinderen een vragenlijst meegestuurd, die ze zelfstandig kunnen invullen.

Wij verzoeken u vriendelijk alle buisjes en de ingevulde vragenlijst mee te nemen naar de afspraak op het UMC Utrecht. Mocht u met de auto komen, dan kunt u parkeren op de parkeerplaats van het UMC Utrecht (zie routebeschrijving).

Mocht u nog vragen hebben dan kunt u mij overdag bereiken op het volgende nummer: 020-598 8820. U kunt ook e-mailen naar ilc.van.soelen@psy.vu.nl.

Met vriendelijke groet,

Inge van Soelen

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

Nederlands Tweelingen Register (NTR)

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Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije* Universiteit *amsterdam

Beste ,

Allereerst heel erg bedankt dat je mee wilt helpen aan ons onderzoek.

Nu gaat het eerste gedeelte van het onderzoek beginnen. Je gaat speeksel, ochtendurine en een monduitstrijkje van jezelf verzamelen. In deze envelop vind je de gebruiksaanwijzingen, waarin staat uitgelegd waarom dit belangrijk is. Ook staat daarin uitgelegd hoe en wanneer je het moet doen. Verder vind je in deze envelop de buisjes die je hiervoor nodig hebt.

Ik wil even wat extra uitleggen over de buisjes met de watjes. Nadat je op het watje heb gekauwd, kan je het watje weer terug doen in de plastic houder (soort van bakje) waar het watje eerst ook in lag. Daarna kan je het samen weer terug doen in het buisje om het zo af te sluiten.

Dit keer willen we ook vragen of jullie een vragenlijst willen invullen. Deze lijst kun je zelfstandig thuis invullen en meebrengen naar de afspraak in Utrecht.

Lees de aanwijzingen goed door. Als je iets niet helemaal begrijpt, vraag het dan aan je ouders of bel mij overdag (Inge: 020-598 8820). Het is de bedoeling dat je alles verzamelt op 2 doordeweekse dagen (met 1 dag ertussen) in de week voordat je naar het UMC Utrecht gaat en het dan meebrengt naar Utrecht.

Heel veel succes!

Met vriendelijke groet,

Inge van Soelen

Afdeling Biologische Psychologie

Bezoekadres: Van der Boechorststraat 1
Transitorium

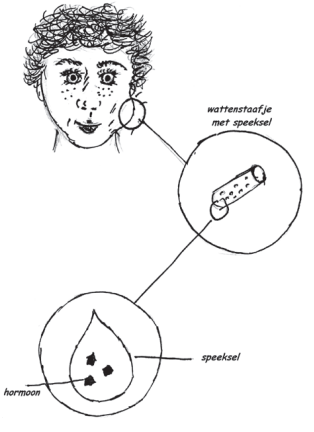
INFORMATIEFOLDER KINDEREN

Tweelingonderzoek naar de ontwikkeling van leren, geheugen en hersenen

✔ Speeksel- en urineverzameling

HORMONEN

Zoals jullie in de eerste folder al gelezen hebben, willen we in dit onderzoek weten of jullie al in de puberteit zijn. Een van de manieren om hier achter te komen is door te kijken hoeveel hormonen jullie in je lichaam hebben. Hormonen zorgen dat je lijf zo verandert dat je volwassen wordt.



Deze hormonen zitten ook in je spuug (speeksel) en plas (urine). Voor het eerste gedeelte van dit onderzoek vragen wij jullie op wattenrolletjes te kauwen en in een potje te spugen. Met behulp van het speeksel dat in deze rolletjes achterblijft en wat je uitgespuugd hebt, kunnen wij onderzoeken hoeveel hormonen jullie in je lichaam hebben. Hieronder staat uitgelegd hoe jullie het speeksel moeten verzamelen. Lees de folder goed door en laat hem ook door jullie ouders lezen.

SPEEKSELVERZAMELING

GEBRUIKSAANWIJZING

WANNEER?

Verzamel het speeksel op twee doordeweekse dagen voorafgaand aan de dag dat jullie naar het UMC Utrecht komen om de testen te gaan doen. Tussen de twee dagen dat je het speeksel gaat verzamelen, zit één dag waarop je niet meet. Daarnaast is het belangrijk dat jij en je broer(s) en/of zus(sen) ongeveer tegelijkertijd het speeksel verzamelen.

HOE LAAT?

1. direct als je wakker wordt, als je nog in bed ligt
2. 15 minuten na het wakker worden
3. 30 minuten na het wakker worden
4. 45 minuten na het wakker worden
   Nu kun je ontbijten.
5. 12.00 uur (voor het middageten)

HOE?

1. Als je 's morgens wakker wordt, willen we dat je niet alleen op het wattenrolletje kauwt, maar ook dat je op een theelepeltje spuugt (alle andere keren van de dag hoef je alleen nog op het wattenrolletje te kauwen). Laat het speeksel uit je mond op het lepeltje lopen. Giet het speeksel van af het lepeltje in het buisje (zonder watje, met de juiste sticker) en doe het dopje er goed op. **Verzamel tenminste 2 cm speeksel!** (Zorg bij het bewaren dat het buisje rechtop staat.) Nadat je dit gedaan hebt, kauw je op het wattenrolletje.

2. Voor het kauwen op het wattenrolletje willen we dat je op de hierboven en op de buisjes aangegeven tijdstippen het buisje met het wattenrolletje uit de houder neemt. Het tijdstip dat op de sticker van de houder staat, moet hetzelfde zijn als het tijdstip waarop je het speeksel verzamelt.
3. Je draait de dop van de buis en haalt het wattenrolletje eruit.
4. Dan leg je het wattenrolletje ten minste 45 seconden in je mond. Het speeksel gaat door de gaatjes in het plastic hulsje in het wattenrolletje zitten. Het is erg belangrijk (!) dat het wattenrolletje goed nat wordt. Je kunt hierbij helpen door niet te slikken en goed op het wattenrolletje te blijven kauwen en het rolletje heen en weer te bewegen in je mond van links naar rechts en van boven naar beneden.
5. Stop het wattenrolletje terug in de genummerde buis en draai de dop erop.
6. Vul op het schema in de binnenkant van deze folder in hoe laat je verzameld hebt en of je 30 minuten of korter van te voren gegeten, gedronken (water mag wel) en of je je tanden gepoetst hebt. Vul ook in hoe laat je de dagen van de verzameling bent opgestaan en hoe laat je gewoonlijk opstaat.
7. Bewaar de gebruikte genummerde buizen (zowel de buisjes met alleen speeksel, als de buisjes met de wattenrolletjes) in de koelkast.
8. Twee dagen later doe je precies hetzelfde.
9. Neem de buisjes mee naar de afspraak in Utrecht.

Haal het hele middenvel uit de folder, vul het schema (zie punt 6 op bladzijde 6 van de folder) in en neem het mee naar je afspraak op de VU.

Tijdstippen van:	Hoe laat heb je het speeksel verzameld?	Heb je 30 minuten van tevoren gegeten /gedronken/tanden gepoetst?
Speekselverzameling dag 1		
Direct na het ontwaken		
15 minuten na het ontwaken		
30 minuten na het ontwaken		
45 minuten na het ontwaken		
12.00 uur		
Speekselverzameling dag 2		
Direct na het ontwaken		
15 minuten na het ontwaken		
30 minuten na het ontwaken		
45 minuten na het ontwaken		
12.00 uur		

Naam: .....

Ntrid:.....

Op welke dagen heb je verzameld?

Dag 1 .....

Dag 2 .....

Hoe laat ben je op deze dagen opgestaan?

Dag 1 .....uur

Dag 2 .....uur

Hoe laat sta je gewoonlijk op doordeweeks?

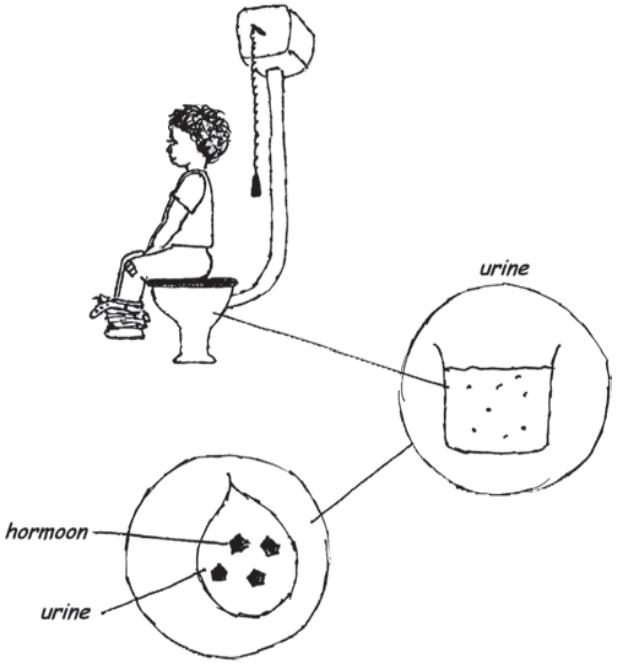
.....uur

ETEN EN DRINKEN

Verder is het erg belangrijk dat je een half uur voordat je op het wattenrolletje kauwt, niets hebt gegeten en geen koffie, thee of fris hebt gedronken. Het is het beste als je pas je ontbijt neemt als je klaar bent met het verzamelen van het ochtendspeeksel. Dit is omdat er anders broodkruimels en ander eten in het speeksel gaat zitten. Je kunt wel gewoon water drinken. Verder is het belangrijk om niet vlak voor het speeksel verzamelen je tanden te poetsen (anders kan er misschien bloed in je speeksel terecht komen). Het beste kun je elke keer voordat je speeksel verzamelt, even kort je mond spoelen met water.
Mocht het nu echt niet lukken om tussendoor niet te eten, dan vragen we je tenminste tot nà de derde keer van het speeksel verzamelen te wachten met ontbijten. Eet en drink dan geen zure voedingswaren, zoals fruit, yoghurt of priklimonade.

OCHTENDURINE

Voor het tweede gedeelte van dit onderzoek vragen wij jullie om ochtendplas te verzamelen. Met behulp van jullie ochtendplas kunnen wij onderzoeken hoeveel hormonen jullie in je lichaam hebben. Hieronder staat uitgelegd hoe jullie dat moeten doen. Lees het onderstaande verhaal goed en laat hem ook door jullie ouders lezen.



GEBRUIKSAANWIJZING

WANNEER?

Verzamel de ochtendplas op **twee** doordeweekse dagen in de week voorafgaand aan de afspraak op het UMC Utrecht. Doe dit op dezelfde dagen als je je speeksel verzamelt.

HOE?

De eerste keer dat je 's morgens moet plassen, plas je in plaats van in de wc-pot in een schoon potje (bijvoorbeeld een schoongemaakt jampotje). Giet de urine over in het buisje met het juiste etiket. Vul het buisje voor 3/4. Draai daarna het dopje goed op het buisje. Bewaar de buisjes rechtop in de koelkast. Neem de buisjes mee naar de afspraak in Utrecht.

VRAGEN

Als je nog vragen hebt, kun je contact met mij opnemen. Mijn gegevens staan op de achterzijde van deze folder.

Veel succes!

INSTRUCTIE

HET MONDUITSTRIJKJE:
hoe verzamel je DNA
met een wattenstaafje

VRIJE UNIVERSITEIT
NEDERLANDS TWEELINGEN REGISTER

NEDERLANDS TWEELINGEN REGISTER

Waarom monduitstrijkjes?

Met behulp van tweelingen en hun families onderzoekt het Nederlands Tweelingen Register de invloed van erfelijke factoren en omgevingsfactoren op individuele verschillen in gedrag en gezondheid. Door middel van vragenlijst- en laboratoriumonderzoek kan worden nagegaan hoe groot deze erfelijke en omgevingsinvloeden zijn. Als er erfelijke invloeden zijn, kan met deze methoden echter niet bepaald worden welke genen verantwoordelijk zijn voor verschillen in gedrag en gezondheid. Daarvoor is erfelijk materiaal, het zogenaamde DNA, nodig.

Met een monduitstrijkje kan op een eenvoudige en pijnloze manier erfelijk materiaal worden verzameld.
Een monduitstrijkje wordt gemaakt door met een wattenstaafje zachtjes langs de binnenkant van de mond te wrijven. De cellen van het wangslijmvlies worden zeer vaak vernieuwd. Daarom zijn deze cellen bij uitstek geschikt voor verzameling van erfelijk materiaal.
De cellen kunnen ook worden gebruikt om te bepalen of een tweeling één- of twee-eiig is. De tweelingen die aan dit onderzoek meedoen krijgen deze uitslag te zijner tijd thuisgestuurd.

INSTRUCTIE

Wat zit er in de enveloppe?

Je hebt 5 buisjes ontvangen: een wat dikkere buis met 16 wattenstaafjes en 4 dunne buisjes met alleen een beetje vloeistof.

Wat te doen met het materiaal?

De monduitstrijkjes moeten 4 keer worden afgenomen, verspreid over twee dagen (2 keer per dag; bijv. voor de ochtend- en avondmaaltijden). Niet van te voren de mond spoelen, tanden poetsen of eten!

Per afname gebruik je 4 wattenstaafjes:

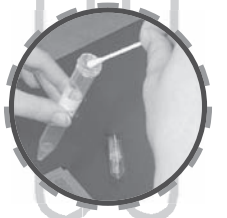
- 1 staafje voor de binnenkant van de bovenlip en het tandvlees van de bovenkaak.
- 1 staafje voor de binnenkant van de onderlip en het tandvlees van de onderkaak.
- 1 staafje voor de binnenkant van de linkerwang.
- 1 staafje voor de binnenkant van de rechterwang.

Je wrijft per wattenstaafje ongeveer 10-20 seconden zorgvuldig en met enige druk (het hoeft niet hard; het mag geen pijn doen).

Na het wrijven deponeer je het staafje, met het watje naar beneden, in de vloeistof in één van de dunne buisjes. Doe alle 4 staafjes in ditzelfde dunne buisje. De andere dunne buisjes zijn voor de volgende monduitstrijkjes. Wil je het deksel van het buisje goed dichtdraaien? (Niet te hard om barsten van de buis te voorkomen).
Als je na twee dagen alle vier de afnames hebt gedaan, heb je de dikke buis niet meer nodig. Deze kun je dus weggooien.

Nadat het erfelijk materiaal is geanalyseerd, zullen eventuele restanten voorlopig opgeslagen worden voor mogelijk aanvullende bepalingen ten behoeve van het onderzoek naar leefgewoonten, gedrag en gezondheid. Wanneer je dit niet wilt, stel ons dan daarvan s.v.p. op de hoogte. In dat geval zullen wij het overgebleven materiaal vernietigen.

Indien je nog vragen hebt, kun je contact opnemen met ons secretariaat. Onze gegevens staan op de achterzijde van deze brochure.



NEDERLANDS TWEELINGEN REGISTER

DAGPROGRAMMA

UMC UTRECHT

Ochtend

Na aankomst bij de hoofdingang van het UMC Utrecht kunt u zich melden bij de receptie. Een van de medewerkers van dit onderzoek haalt u op bij de hoofdingang.

Eenmaal bij de testkamers aangekomen krijgt u uitleg over het dagprogramma. U kunt de vragenlijsten, de buisjes met speeksel, ochtendurine en de monduitstrijkjes die thuis zijn afgenomen inleveren.

Vervolgens wordt er met het onderzoek begonnen. Afwisselend worden verschillende soorten taken gedaan. Sommige taken bestaan uit het maken van puzzels, andere taken bestaan uit het beantwoorden van vragen. De taken worden van tevoren allemaal duidelijk uitgelegd. Afwisselend worden er taken op de computer of met pen en papier gedaan. Ook de ouders wordt gevraagd om een soortgelijke taak als de kinderen uit te voeren.

Tussen de testen door wordt er een korte pauze gehouden, waarin iets te drinken en een versnapering wordt aangeboden.

Middag

Na de lunch, die u door het UMC krijgt aangeboden, zal er verder gegaan worden met het leervermogenonderzoek. Als laatste onderdeel van de dag zal de MRI-scan volgen. Voordat er een MRI-scan wordt gemaakt van de hersenen, krijgen de kinderen een zorgvuldige uitleg over de scanprocedure en kunnen ze alle handelingen oefenen in de speciaal daarvoor bestemde oefenscanner. Dit is een oefenapparaat dat precies hetzelfde is als de echte scanner maar waar niet mee gescand wordt. De kinderen kunnen dan wennen aan de kleine ruimte en aan de geluiden.

Vervolgens kunnen ze de metalen voorwerpen (bijvoorbeeld horloges en oorbellen) die zij nog bij zich dragen afdoen in de hiervoor bestemde kleedruimte (persoonlijke eigendommen kunnen veilig worden opgeborgen). Om er zeker van te zijn dat de kinderen geen metalen voorwerpen meer bij zich dragen op het moment dat zij de scanruimte ingaan worden hierover door de onderzoeker nog enkele gerichte vragen gesteld.

De totale scanprocedure zal per kind ongeveer drie kwartier in beslag nemen. Uw kind staat via een microfoon en een alarmbel in contact met de onderzoekers. Het is belangrijk te weten dat op ieder moment de kinderen kunnen aangeven te willen stoppen met het onderzoek. Ook is het mogelijk dat een van de ouders mee de onderzoeksruimte in gaat. Gedurende de MRI-scan hoeven de kinderen geen taken uit te voeren en wordt hen gevraagd zo stil mogelijk te liggen (afgezien van enkele korte pauzes). Ter ontspanning kunnen ze naar een tekenfilm (eventueel een zelf meegebrachte DVD) kijken of naar muziek luisteren.

Tijdens het wachten op elkaar voor de MRI-scan krijgen de kinderen een kort fysiek onderzoek naar de lichamelijke ontwikkeling, waarvoor ze zich zullen moeten uitkleden (zie informatiefolder) en zal er een korte leesvaardigheidtest worden afgenomen.

Na afloop van het onderzoek krijgen de kinderen een tegoedbon om zelf een leuk cadeautje uit te zoeken. De ouders/verzorgers krijgen een reiskostenvergoeding.

Z.O.Z.

CHECKLIST UMC UTRECHT

Heeft u het volgende bij u?

- Uw monduitstrijkjes en die van uw kinderen
- Speeksel van uw kinderen
- Ochtendurine van uw kinderen
- Vragenlijsten ingevuld door uw kinderen *(Deel I)*
- Vragenlijsten ingevuld door u zelf
- Burger service nummer van uw kinderen (vroegere sofinummer, staat ook vermeld op de zorgpassen van de kinderen)
- Groei boekjes (groene boekje), als u deze nog in uw bezit heeft.

Als u een van de volgende vragen bevestigend beantwoordt, zou u dan contact met mij willen opnemen?

(ilc.van.soelen@psy.vu.nl of 020-598 8820)

- Heeft uw kind een metalen beugel in de mond die niet te verwijderen is?
- Heeft uw kind een pacemaker- of hartklepoperatie ondergaan?
- Heeft uw kind clips in de bloedvaten in het hoofd?
- Heeft uw kind metaalsplinters (onverwijderd) in zijn/haar hoofd?
- Is uw kind angstig in nauwe ruimtes?
- Heeft uw kind een hydrocephalus- of een insulinepomp die niet te verwijderen is?
- Zijn er stents in het lichaam van uw kind geplaatst?
- Heeft uw kind metalen implantaties, oor- of oogprotheses? (b.v. piercings, gewrichtsvervangingen, schroeven, cavafilters)
- Heeft uw kind een gehoorapparaat dat niet te verwijderen is, of een of meer metalen oorbuisjes?
- Is er bij uw kind een port-a-cath catheter aangeprikt?

Tweelingonderzoek naar de ontwikkeling van brein en cognitie tijdens de pre-adolescentie

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 "**Tweelingonderzoek naar de ontwikkeling van brein en cognitie tijdens de pre-adolescentie**" 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 *zelf* deel te nemen aan het onderzoek:

Toestemming voor:

* deelname aan het cognitie-onderzoek	0 ja	0 nee
* deelname aan het DNA-onderzoek en de opslag en analyse van het erfelijk materiaal	0 ja	0 nee

Naam: ……. man/vrouw

Geboortedatum: …….

Straat en huisnr.: …….

Postcode en plaats: …….

Telefoonnummer: …….

Handtekening: …… Datum: ……..…

Ik, ondergetekende, bevestig hierbij dat deze studie zowel mondeling als schriftelijk aan bovengenoemde deelnemer is uitgelegd.

Naam arts/onderzoeker: ……

Handtekening: …….. Datum: ……..…….

Tweelingonderzoek naar de ontwikkeling van brein en cognitie tijdens de pre-adolescentie

verklaring van toestemming na kennisneming

voor de tweeling / broer / zus

Wil je hieronder tekenen en daarmee het volgende verklaren:

1) De onderzoeker heeft mij volledig ingelicht over de aard en het doel van het "**Tweelingonderzoek naar de ontwikkeling van brein en cognitie tijdens de pre-adolescentie**" 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 hormoononderzoek op basis van speeksel en urine ja/ nee
* het MRI onderzoek (hersenfoto's) ja/ nee
* de cognitieve en neuropsychologische testen ja/ nee
* opslag en analyse van het erfelijk materiaal (DNA) ja/ nee
* ik wil mijn testresultaten graag thuisgestuurd krijgen ja/ nee
* na afloop van dit onderzoek mag ik weer benaderd worden voor vervolgonderzoek ja/ nee

Naam kind: …… jongen/meisje

Geboortedatum: ……

Straat en huisnr.: ……

Postcode en plaats: ……

Telefoonnummer: ……

Handtekening: …… Datum: ……..

Naam ouder/vertegenwoordiger: …….

Handtekening: …… Datum: ……..…..

Naam (eventuele) tweede ouder/vertegenwoordiger: ……..

Handtekening: …… Datum: ...……

Ik, ondergetekende, bevestig hierbij dat deze studie zowel mondeling als schriftelijk aan de bovengenoemde deelnemer is uitgelegd.

Naam arts/onderzoeker: ……

Handtekening: …… Datum: ……...

List of Publications

List of Publications

I.L.C. van Soelen, R. M. Brouwer, G.C.M. van Baal, H.G. Schnack, J.S. Peper, D.L. Collins, A.C. Evans, R.S. Kahn, D.I. Boomsma, H.E. Hulshoff Pol. **Genetic influences on thinning of the cerebral cortex during development.** *(In revision)*

I.L.C. van Soelen, R. M. Brouwer, G.C.M. van Baal, H.G. Schnack, J.S. Peper, L. Chen, R.S. Kahn, D.I. Boomsma, H.E. Hulshoff Pol. **Heritability of volumetric brain changes and height in children entering puberty.** *(Accepted for publication; Human Brain Mapping)*

I.L.C. van Soelen, R. M. Brouwer, M. van Leeuwen, R.S. Kahn, H.E. Hulshoff Pol, D.I. Boomsma (2011). **Heritability of verbal and performance intelligence in a longitudinal pediatric sample.** *Twin Research and Human Genetics, 14(2), p 119-128.*

I.L.C. van Soelen, R.M. Brouwer, J.S. Peper, T.C. van Beijsterveldt, M. van Leeuwen, L.S. de Vries, R.S. Kahn, H.E. Hulshoff Pol, D.I. Boomsma (2010). **Effects of Gestational Age and Birth Weight on Brain Volumes in Healthy 9 Year-Old Children.** *Journal of Pediatrics, 156(6), p 896-901*

I.L.C. van Soelen, S.M. van den Berg, P.H. Dekker, M. van Leeuwen, J.S. Peper, H.E. Hulshoff Pol, D.I. Boomsma (2009). **Individual differences in dynamic measures of verbal learning abilities in young twin pairs and their older siblings.** *Learning and Individual Differences, 19(4), p 440-444.*

D. van 't Ent, **I.L.C. van Soelen**, C.J. Stam, E.J. de Geus, D.I. Boomsma (2010). **Genetic influence demonstrated for MEG-recorded somatosensory evoked responses.** *Psychophysiology, 47(6), p 1040-1046*

D. van 't Ent, **I.L.C. van Soelen,** C.J. Stam, E.J. de Geus, D.I. Boomsma (2009). **Strong resemblance in the amplitude of oscillatory brain activity in monozygotic twins is not caused by "trivial" similarities in the composition of the skull.** *Human Brain Mapping, 30(7), p 2142-5*

Published abstracts

I.L.C. van Soelen, R.M. Brouwer, G.C.M. van Baal, H.G. Schnack, J.S. Peper, R.S. Kahn, D.I. Boomsma, H.E. Hulshoff Pol. Heritability of pre-adolescent brain development: a longitudinal twin study. *(Behavior Genetics, 2010)*

I.L.C. van Soelen, R.M. Brouwer, G.C.M. van Baal, H.G. Schnack, J.S. Peper, R.S. Kahn, D.I. Boomsma, H.E. Hulshoff Pol. Genetic Influences on Brain Morphometry and Developmental Changes in Pre-adolescence. *(Human Brain Mapping, 2010)*

I.L.C. van Soelen, R.M. Brouwer, J.S. Peper, C.E.M. van Beijsterveldt, M. van Leeuwen, R.S. Kahn, H.E. Hulshoff Pol, D.I. Boomsma. The Influence of Pregnancy Duration on Cerebellum Volume in Healthy 9-year-old Twin Pairs. (*Organization of Human Brain Mapping 2009 Annual meeting). NeuroImage (47) supplement 1*

I.L.C. van Soelen, S.M. van den Berg, P.H. Dekker, M. van Leeuwen, D.I. Boomsma. Genetic and environmental influences on verbal learning abilities in children. *Behavior Genetics, 2007 (36).*

Dankwoord

Dankwoord

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