# Adolescent brain development

A longitudinal twin study into structural brain development and its relation to hormone levels and intelligence

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**Hersenontwikkeling tijdens adolescentie**

Een longitudinale tweeling studie naar de ontwikkeling van hersenstructuur en de relatie met hormoonspiegels en intelligentie (met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 6 juli 2017 des middags te 12.45 uur

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Prof. dr. D.I. Boomsma

Co-promotor: dr. R.M. Brouwer

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Chapter 1

# Introduction

## GENERAL INTRODUCTION

Adolescence is defined as the 'period following the onset of puberty during which a young person develops from a child into an adult' (Oxford Dictionary). Puberty is the period during which a person reaches sexual maturity and becomes capable of reproduction. Thus, adolescence relates to the cognitive and behavioral changes that shape a child into an adult and puberty to the development of secondary sexual characteristics. Combined, they describe a period during which major changes take place.

Although the exact mechanisms that trigger the start of puberty are not known, once the first nightly increases in levels of luteinizing hormone (LH) have occurred, the cascade of hormonal and physical changes are relatively clear and straightforward. The teenage brain undergoes considerable reorganization on a structural and functional level. These changes have been associated with cognitive and social development. Because of these changes, adolescence is also a precarious period, with a number of developmental psychiatric disorders having their onset during this period.

Determining the process of brain development during puberty is thus not only important for understanding the individual differences in development of teenagers but will also for understanding the causes and consequences of developmental disorders. Both genes and environmental factors influence brain development. Healthy brain development and discovering to which extent genetic and environmental factors influence healthy brain development is one of the aims of the BrainSCALE project on which this dissertation is based (see also van Soelen et al., 2012).

The BrainSCALE project is a longitudinal study that currently encompasses three assessments in a large group of twins and siblings at ages 9, 12, and 17 of the twins. Data collection included IQ (intelligence quotient) tests, comprehensive neuropsychological testing protocol, parental and self-ratings of behavioral and emotional problems, physical development, hormonal levels, and MRI (magnetic resonance imaging) scans of the brain. The data of the first assessment has been the basis of the dissertations of Marieke van Leeuwen (van Leeuwen, 2008) and Jiska Peper (Peper, 2008). The second assessment has been the basis of the dissertation of Inge van Soelen (van Soelen, 2011). This dissertation is based on the data of the first, second, and third measurement.

In this dissertation, I specifically examine hormonal development and how that relates to brain development (measured with partial volume density) and changes in gray matter density; and white matter connectivity and how that relates to intelligence. In addition, I investigate to what extent genes influence these variables and their relations. This chapter introduces the many aspects of this study and concludes with an overview and background of the research questions.

## HORMONAL INFLUENCES

Because the adolescent period is characterized by both major hormonal and brain changes, the question has risen how these two are linked. Here, I first describe the start of puberty and the function of the main four sex hormones LH (luteinizing hormone), FSH (follicle stimulating hormone), estrogen and testosterone. Then I will give a short overview of literature on the relation between these hormones and the brain.

Although the exact trigger of the onset of puberty is still unknown, the steps of the reac-

tivation of the HPG-axis (hypothalamic-pituitary-gonadal-axis) are clear (review by Marceau et al., 2015). It is a reactivation because the HPG axis is also active in the perinatal period. Once the hypothalamus starts to excrete gonadotropin-releasing hormone (GnRH), this hormone in turn signals the pituitary gland to secrete nightly surges of the gonadotropins LH and FSH. The nightly LH surges occur 1-2 year before the onset of secondary sexual characteristics. LH and FSH stimulate the gonads (testes in boys, ovaries in girls) to produce testosterone (by the Leydig cells in testes) and estradiol (by the ovaries). Testosterone and estradiol are present in both boys and girls due to conversion of androgens (produced by the adrenal gland and gonads) and cholesterol.

Sex hormones are required for the onset of secondary sexual characteristics such as pubic hair development, breast development, penis and testes enlargement, and onset of menstruation (menarche). These physical changes occur 1-2 years earlier in girls (typically around age 10-11) than in boys (typically around age 11-12) (Marshall and Tanner, 1969; Marshall and Tanner, 1970; Mul et al., 2001). Behavioral changes like increased sexual drive and risk taking during puberty have been suggested to be linked to increased hormone levels (Braams et al., 2015; Op de Macks et al., 2016; Schulz and Sisk, 2016). Sex steroid (testosterone and estrogen) receptors are present in many regions of the brain and mounting evidence indicates a relation between steroid hormones and spatial problem solving, verbal abilities, aggression, motor activity, learning and memory and affect regulation (see reviews by Hara et al., 2015; McEwen and Alves, 1999). Therefore, the rise in hormone levels during puberty may be related to behavioral changes and to brain development.

**THE ADOLESCENT BRAIN**

Puberty is not only a period of considerable changes in hormone levels and physical appearance, but also of dynamic changes in the brain. On a global scale, the major developmental changes in the teenage brain are a profound decrease in gray matter volume (the neuronal cell bodies) and a continuous increase in white matter volume (the axonal fiber connections between the cells). Gray matter volume peaks between the ages 8-16 – depending on the region – after which it decreases (Giedd et al., 1999; Gogtay et al., 2004; Raznahan et al., 2011b; Wierenga et al., 2014). White matter volume (Hedman et al., 2012) continues to increase approximately age 45; and white matter integrity until approximately age 35 (Lebel et al., 2012; Yap et al., 2013). In addition to these global dynamics, the developmental progression differs across brain regions. Thus, increases and decreases in gray matter volume take place at the same time, along with an increase in white matter volume, which is also not uniform over the brain. Furthermore, peak volume of gray matter is reached 1-2 years earlier in girls than in boys (Giedd et al., 1999; Raznahan et al., 2011a), echoing the onset of puberty (Mul et al., 2001).

The dynamic changes in the brain are considered to be related to changes in (social) behavior associated with adolescence. For example, subcortical and cortical regions develop at a different age (Sowell et al., 1999), resulting in a (functional) imbalance that is further enhanced by immature network of connections between subcortical, limbic, and prefrontal brain regions. The imbalance between partly mature, partly immature brain regions is thought to be the cause of teenage impulsivity and reward-biased behavior (Casey, 2015; Crone and Dahl, 2012; Somerville et al., 2010).

The considerable reorganization of the brain also makes the teenage period sensitive to developmental perturbations that could lead to increased risk of onset of psychiatric diseases such as schizophrenia, anxiety and mood disorders, eating disorders, and substance abuse (Insel, 2010; Paus et al., 2008). It is therefore important to study typical development of the teenage brain; one of the main aims of the BrainSCALE study. My specific focus lies on the network of connections in the brain: how do they develop during this dynamic period?

**BRAIN IMAGING**

In this thesis, the developing teenage brain was studied with brain images acquired with a Magnetic Resonance Imaging (MRI) scanner. With an MRI scanner, detailed images of the gray and white matter can be obtained as well as microstructural properties of white matter and brain activity during rest or a task. An MRI scanner consists of a strong magnet and uses the tiny magnetic property of protons (specifically those of hydrogen atoms) to image the inside of the body. This small magnetic field is called the nuclear spin. In the MRI scanner, the protons have the tendency to align with the magnetic field created by the magnet. When making an image, an electromagnetic pulse, the radio frequency (RF) pulse, is given to excite the protons away from their equilibrium (or 'ground') state. When the RF pulse stops, the protons want to align back with the magnetic field. After a certain time, the protons fall back to their equilibrium state (this is called relaxation). As the protons fall back, they emit an RF pulse that is recorded by the receiving coil. The contrast between different tissues is determined by the rate at which excited protons return to the equilibrium state. This gives the well-known gray-scale pictures of the brain; the T1-weighted image. In this image, we can differentiate between gray matter, white matter and cerebral spinal fluid.

***Brain volumes and densities***

An MRI image is a three-dimensional picture that consists of voxels. A voxel is a volume element that can be describes as a three-dimensional pixel. Each voxel has a signal intensity value that can be used to separate gray matter, white matter and cerebrospinal fluid. By counting the number of voxels containing a specific tissue type (taking the size of the voxels and partial voluming (Brouwer et al., 2010) into consideration) the volume of the total brain, gray matter, white matter and cerebrospinal fluid can be determined. To study group differences in the brain at a local level, voxel-based morphometry (VBM; Ashburner and Friston, 2000) can be used to perform a voxel-by-voxel comparison.

***Diffusion Tensor Imaging***

The microstructure of white matter can be (indirectly) measured with Diffusion Tensor Imaging (DTI). The basis of DTI is the random motion of water molecules. If water molecules are given free reign, they move randomly (Brownian motion) but roughly evenly in all directions. This is called isotropic diffusion (from Greek *isos*, "equal", and *tropos*, "way"), which indicates that movement of water molecules is equal in all directions. However, when the diffusion is more restricted in one direction than the other (typically in white matter fiber bundles where water molecules move more easily along the axons that form the bundle than across the bundle) the diffusion profile of the water molecules is anisotropic. This preferred movement of water molecules along the axons can inform us of the direction of the white matter bundle.

Fractional anisotropy (FA) is a frequently used scalar measure that reflects the level of anisotropy of the diffusion profile and ranges from 0 (isotropic diffusion) to 1 (completely anisotropic diffusion). In white matter, various elements contribute to the shape of the diffusion profile including alignment and packing density of the axons, axonal diameter, and myelination (for more detail, see Beaulieu, 2002). As such, FA is often considered as an index of white matter integrity.

***White matter pathways***

The brain is an interconnected system with different functions that run in parallel and regions that continuously communicate with each other through white matter bundles. The quality of a white matter bundle that connects two regions can be assessed with FA, or streamline-count. Streamline-count refers to the number of 'lines' that can be reconstructed between two regions. Although it may appear as if the number of streamlines is a proxy for the number of axonal connections, it has no direct physiological meaning and cannot be interpreted as such. Because FA is used as a guide for the reconstruction process of streamlines, streamline count is also related to density of fiber tracts, alignment of the fibers, axonal diameter, and myelination. However, streamline count is more affected by length, curvature and branching of white matter bundles (Jones et al., 2013).

FA increases during the whole period of development (from birth to ~ 35 yr; Lebel et al., 2012; Yap et al., 2013). This is caused by alignment of individual axons, their packing density and myelination, all of which are thought to promote the efficacy of neural communication and increase FA (Paus, 2010). Because white matter bundles do not all mature at the same time (Lebel and Beaulieu, 2011; Schmithorst and Yuan, 2010; Wierenga et al., 2016) and because the white matter network is important for integration and processing of information, a network approach to the development of white matter bundles was used in this thesis.

***The brain as a network***

To study the network of all connections and determine the communication efficiency of the white matter network as a whole, we used graph theory. When one views the brain as a network (on a macroscale), brain regions can be denoted as vertices or nodes, and the connections between the brain regions (the white matter bundles in our case) as edges. With mathematics, one can describe properties of this network, like information transfer on a local scale and on a global scale.

Earlier studies (Achard and Bullmore, 2007; van den Heuvel et al., 2008) have shown that the brain can be described as a small world network. This term has been coined by (Watts and Strogatz, 1998). In their study, they showed that if you randomly rewire only a few edges of a regular or lattice-like network, you get a network that has good neighbor-to-neighbor communication, but also good long distance communication due to those few long edges. Neighbor to neighbor communication is called local efficiency; it reflects how well information can travel in the direct neighborhood of a node, and is often interpreted as a measurement of the local information processing capacity of a network. Global efficiency is a network characteristic that quantifies how easy information can be exchanged over the entire network, providing information on the communication efficiency of a network as a

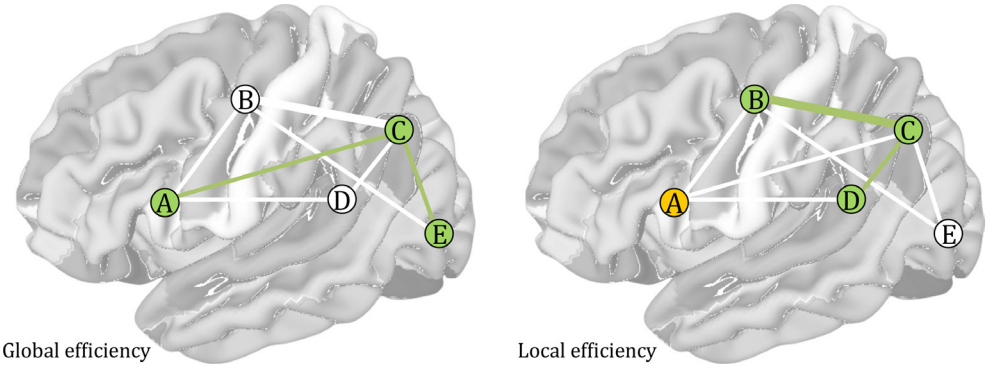


**Figure 1.1** – In this example, the network consists of 5 nodes (A through E). The global efficiency of the network is mean of the inverse of the distance between each pair of nodes. Distance is computed as the shortest paths. As an example, the shortest path from A to E is via C (indicated in green). Local efficiency of node A is the efficiency of the network NA, which contains only the direct neighbors of A, without A itself; thus B, C and D (colored green). Efficiency is then computed as the mean of the inverse distance between all pairs of nodes in the network NA. The paths between the nodes of NA are colored green.

whole (Bullmore and Sporns, 2012; van den Heuvel and Hulshoff Pol, 2010). Many more mathematical descriptions of a network configuration, or topology, have been developed. In this dissertation, we use these two basic definitions (see Figure 1.1).

Network characteristics can be computed from binary networks, where either an edge is present or not, or on weighted networks, where the strength of each edge or connection is taken into account. Here, FA or streamline-count are used as edge-weights. In this way, all the information on connection strength is included in the computations.

**INTELLIGENCE**

Despite the fact that many people would agree that they understand what intelligence is, to find one concluding definition has been proven to be difficult. A definition that is often used is the following: "Intelligence is a very general capability that, among other things, involves the ability to reason, plan, solve problems, think abstractly, comprehend complex ideas, learn quickly and learn from experience. It is not merely book learning, a narrow academic skill, or test-taking smarts. Rather, it reflects a broader and deeper capability for comprehending our surroundings—'catching on', 'making sense' of things, or 'figuring out' what to do." (Gottfredson, 1997).

Intelligence is often measured with the Wechsler Intelligence Scale for Children between the ages 6 through 17 years; in adults between 16 and 85 years, the Wechsler Adult Intelligence Scale is often used. The raw scores on these tasks are translated to a normalized intelligence quotient (IQ) based on somebody's age. This means that an individual's score is compared to the score of the same age group. For each age group, mean IQ is set to 100 with a standard deviation of 15 IQ points. The age groups for children have a smaller range (3 months to a year) than the age groups for adults (roughly 10 years).

In the BrainSCALE study, IQ has been tested with the Wechsler intelligence scales at all three waves. These tests include several aspects of cognitive functioning, like vocabulary,

(verbal & nonverbal) reasoning and spatial ability. Scores on these subtests are positively correlated as a 'general intelligence' factor influences all these cognitive domains (Figure 1 from Deary et al., 2010). This was already discovered in the early 1900s by Spearman (Spearman, 1904).

***Intelligence and the brain***

Can we identify where in the brain intelligence is 'seated' and how differences in intellectual capacity come about? In the late nineteenth and early twentieth century, it was found that people with a larger head circumference had a higher mental ability (see overview in Rushton and Ankney, 2009). Nowadays, brain size still has one of the strongest correlations with intelligence (r=0.33, meta-analysis by McDaniel, 2005, see also review Rushton and Ankney, 2009). This relation is explained by genetic factors that influence both IQ and total brain volume (Posthuma et al., 2002), and local brain volumes (Bohlken et al., 2016; Hulshoff Pol et al., 2006). Many studies focus on the search for regions related to specific cognitive functions. Concerning general intelligence, a detailed review has been written by Jung and Haier (2007). They found that often the same regions were correlated with intelligence, because either of increased volume, activity, or connections between the regions. These regions are located all over the brain; frontal, parietal, temporal and occipital regions, therefore, their conclusion was that a network of regions is implicated in intelligence. More specifically, these brain regions work together to achieve mental performances, hence they coined the term P-FIT, the Parieto-Frontal Integration Theory. Consequently, people with a more efficiently wired brain – based on either functional (Bassett et al., 2009; van den Heuvel et al., 2009; Langer et al., 2012) or structural imaging (Li et al., 2009; Wen et al., 2011) – are often found to be more intelligent.

However, those studies have been performed in adults; it is not known what the relation between IQ and the brain network is in children and adolescents. Adolescence is a period of major brain reorganization and several studies have shown that brain development during this period is related to intellectual abilities (Brouwer et al., 2014; Schnack et al., 2015; Shaw et al., 2006; Tamnes et al., 2010). As such, the brain areas and networks related to IQ may also be a work in progress. In addition, although the functional network of the brain is influenced by genetic factors in children (van den Heuvel et al., 2013) and structural and functional network topology adults (Bohlken et al., 2014; Fornito et al., 2011; Jahanshad et al., 2012), it is not known whether the relative contribution of genetic and environmental factors on the topology of the white matter network changes during development. Besides, during childhood and adolescent development, intelligence becomes more influenced by genes and relatively less by environmental factors (see review Deary et al., 2009). These changes in relative influences of genes and environment to IQ may be related to the relation between IQ and brain changes (Brouwer et al., 2014; Tamnes et al., 2010). With our extended twin design (see next paragraph), we are also able to shine a light on these questions.

**STUDYING TWINS**

Variation in intelligence (and many other traits) is partly due to differences in DNA sequence; the genetic information. In many studies, the twin model is applied to determine to what extent a trait of interest is influenced by genetic or environmental factors. This is

because of the unique feature that identical (or monozygotic, from one fertilized egg; MZ) twins share (nearly always) 100% of their DNA, they are thus genetically the same. Fraternal (dizygotic, from two fertilized eggs; DZ) twins share on average 50% of their segregating genes, like other full siblings. Both MZ and DZ twins share a part of their environment, not only after they are born (same household and neighborhood), but also prenatally as they share the womb. Beside genetic and common environmental factors, the unique environment of an individual (like friends and sports that they do not share with their co-twin) can also contribute to the variation in a trait.

These three factors form the basis of the classical twin design to disentangle the relative contribution of genetic factors, common environmental factors and unique environmental factors to the total variance in a trait. When MZ twins are more similar (i.e., higher correlation between the two members of a twin pair) with respect to a trait – for example height – than DZ twins, this indicates that genes play an important role in this trait. Heritability is then defined as the proportion of the total variance of a trait that is explained by genetic factors. When MZ twins are equally similar as DZ twins, or in other words, the MZ and DZ twin correlations are roughly equal but larger than zero, the common environment is the important contributing factor. When neither MZ nor DZ twins look alike, the unique environment (and measurement error) mainly influences the trait. In general, genetic effects and common and unique environmental effects all contribute to any trait, albeit in different proportions.

In this example, the genetic effects are due to *additive* genetic effects. When a correlation between MZ twins that is more than twice the correlation of DZ twins, this hints at *non-additive* genetic effects. This is because on average only 25% of non-additive genetic effects are shared between DZ twins (compared to the 50% of additive genetic effects), whereas MZ twins share 100% of their non-additive genetic effects.

What are these genetic effects? Everybody has two copies of a gene (called alleles): one from the biological mother, one from the biological father. Some alleles are monomorphic (all alleles are the same) and some alleles are polymorphic and can contribute to phenotypic variation in the population. When the effects of both alleles across all loci that contribute to a trait add up, these effects are called *additive* effects. Non-additive effects can be due to genetic dominance or epistasis. *Dominance* effects refer to interaction between alleles at the same locus and *epistasis* refers to interactions of genes across loci. In human data, these effects are often grouped together as non-additive genetic effects.

For traits that showed evidence for non-additive genetic effects, we estimated the narrow-sense heritability (proportion of the total variance that is explained by additive genetic influences) and the broad-sense heritability (proportion of the total variance that is explained by all genetic influences).

In addition to estimating genetic and environmental influences on one trait, bivariate or multivariate twin data can also be analyzed to estimate the genetic correlation between two (or more) different traits. A genetic correlation, or bivariate heritability, reflects the genetic effects on traits that are correlated. An indication for a high genetic correlation is obtained when for MZ twins, trait A of twin 1 better explains trait B of twin 2 than for DZ twins. In other words, when the cross-trait cross-twin correlations are higher for MZ twins than for DZ twins. An example is the relation between brain volume and IQ: this correlation

is in part due to the same genetic factor that influences both brain volume and IQ (Posthuma et al., 2002).

**OUTLINE OF THIS THESIS**

This thesis captures several aspects of teenage brain development and is part of the BrainSCALE project that was started in 2004. Healthy 9-year old twins and their older siblings had their IQ tested, brains scanned, hormones sampled, and more. This was repeated three years later when the twins were 12 years old. **Chapter 2** provides a description of the participants and data collection of the third measurement (when the twins were 17 years) that took place between October 2012 and December 2013. Results from the third measurement are also reported in the dissertation of Suzanne Swagerman (Swagerman, 2016).

Since hormonal changes are the beginning of puberty, **Chapter 3** explores reproductive hormone levels and their relation to secondary sexual characteristics in 9 and 12 year old twins. At the time of writing chapter 3, surprisingly little was known about normative hormone levels during early adolescence. Besides, because reproductive hormones regulate important changes during puberty, an interesting and important factor of pubertal increases in hormone levels is how genes or environmental factors influence hormone levels during this period of rapid changes. There are some studies that report on the heritability of reproductive hormones in prepubertal boys (Wang et al., 2004), and testosterone in teenagers (Harris et al., 1998; Hoekstra et al., 2006), but there are no reports of the heritability of LH, FSH, and estrogen. In addition to normative hormone levels and their heritability, the relation between hormone levels and secondary sexual characteristics (at the same age and three years later), the presence of a genetic correlation between hormone levels and secondary sexual characteristics (at the same age and three years later) is also explored.

The next question that is addressed, is the relation between hormone levels and brain development. This is explored in **Chapter 4**. Although many studies show a relation between reproductive hormone levels and brain structure during adolescence (Bramen et al., 2011; Bramen et al., 2012; Herting et al., 2012; Herting et al., 2014; Koolschijn et al., 2014; Neufang et al., 2009; Nguyen et al., 2013a; Nguyen et al., 2013b; Peper et al., 2008; Peper et al., 2009; Perrin et al., 2008); see also reviews by (Ladouceur et al., 2012; Peper et al., 2011a; Peper et al., 2011b; Sisk and Zehr, 2005), the results are not easily summarized. For example, associations between testosterone and regional volumes are reported for different brain regions [amygdala (Herting et al., 2014; Neufang et al., 2009), hippocampus (Neufang et al., 2009) ACC and OFC (Koolschijn et al., 2014)]; and it is not clear whether there is a sex difference in the correlation between testosterone and white matter volume [correlation only in boys (Perrin et al., 2008), or in both sexes (Herting et al., 2014)]. A likely explanation for this is the rapid development of hormone levels and brain structure during this period of interest. Therefore, longitudinal data is an important feature to shed light on this matter. In this chapter, the relationship between pubertal hormones and gray matter density is explored in a genetically informative longitudinal design.

In **Chapter 5** the influence of genes on the development of white matter network connectivity and its relation with intelligence are studied. Previous studies have shown that (large) white matter bundles show an increase in FA during adolescence (Lebel and Beaulieu, 2011); and review by (Schmithorst and Yuan, 2010), but not all at the same time or pace

(Wierenga et al., 2016). The efficiency of the white matter network changes in the first few decades of life (Dennis et al., 2013; Hagmann et al., 2010; Lim et al., 2015). However, these studies examined a broad age range and the results are not in consensus. Since adolescence is a dynamic period concerning brain changes, a study examining a broad age range might miss important developmental steps that take place during adolescence. Besides, one can imagine that the variation between individuals' brains is substantially increased during adolescence due to (additional) variation in pubertal timing and the rapid dynamics of changes in the brain. Therefore, it is noteworthy to study a small developmental window in a longitudinal study to detect changes during adolescence.

A more efficient white matter network is associated with higher intelligence levels in adults (Li et al., 2009) and the elderly (Wen et al., 2011), but it is not known if that relation is already present at early age when the brain is still developing. Because of our twin sample we are able to test if the relation between network efficiency and IQ is due to genetic or environmental factors that influence both traits. A genetic correlation between FA and IQ has been reported in young adults (Chiang et al., 2009; Chiang et al., 2011a; Chiang et al., 2012). However, as genetic influences on FA are dependent on both age and IQ (Chiang et al., 2011b), the genetic influences on the relation between FA-based network and IQ may also differ over time.

Chapter 6 studies the development of the white matter network and IQ in more depth, with the addition of the third measurement of BrainSCALE at age 17 of the twins. With three measurements, we are able to explore nonlinear developmental patterns over a relatively short period. In addition, we can examine how IQ influences the development of the white matter network. There is only one study that shows that development of FA is related to the level of verbal intelligence (Tamnes et al., 2010), whereas there are numerous studies that report that cortical thickness develops differently depending on IQ (Brans et al., 2010; Brouwer et al., 2014; Schnack et al., 2015; Shaw et al., 2006). In addition, the relative influences of genetic and environmental factors on the white matter network between ages 10 and 18 are investigated.

All results are summarized and discussed in **Chapter 7**.

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Chapter 2

# Sample description and data collection of the 3rd measurement of the brainSCALE study

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## INTRODUCTION

The group of twins and siblings in the BrainSCALE project forms a sample that is followed longitudinally since age 9 of the twins. The BrainSCALE project is a cooperation between the NTR (Netherlands Twin Register) and University Medical Center (UMC) Utrecht, studying the influences on brain and cognition throughout healthy development. Participants were invited for the first assessment in 2004 when the data collections started. This was around the 9th birthday of the twins, and over a 1.5-year period 330 children from 112 families participated. Between 2007-2009 the second assessment took place, this time around the 12th birthday of the twins. In total, 261 children were willing to participate again. Details of the data collection at ages 9 and 12 years are described in the dissertations of Marieke van Leeuwen (van Leeuwen, 2008), Jiska Peper (Peper, 2008) and Inge van Soelen (van Soelen, 2011). An overview of the project was also published in 2012 (van Soelen et al., 2012b). As part of projects in this thesis, twins and siblings were invited for a third assessment. The study was approved by the Central Committee on Research involving Human subjects of the Netherlands (CCMO), and research procedures were performed in accordance with the Declaration of Helsinki. This chapter provides the details of the third wave of data collection that took place in 2012 and 2013.

## SAMPLE CHARACTERISTICS

All families that participated at the first assessment (n=112 families with 224 twins and 96 siblings) were invited to participate again for the third assessment, with the exception of 3 families who had in the meantime indicated that they did not want be approached for follow-up. In total, 246 participants from 89 families agreed to participate (77% return rate). Reasons for not participating were: too busy with school and/or work (21), one or more family members did not want to participate (43), living abroad (5), or unable to reach by mail and phone (3). Of all individuals that did not return at the third assessment, 37 were only part of the first. On the other hand, 31 individuals were not part of the second wave but did decide to return now (see Table 2.1 for number of participants included at the neuropsychological assessment and MRI scans at the three assessments). In total, a large number of participants have participated in all 3 assessments: for 154 participants we have MRI scans and for 212 we have cognitive data on all time points.

Participants in the third assessment consisted of 176 twins (95 female, 81 male) and 70 siblings (44 female, 26 male) from 89 families. Twin pairs were almost equally distributed for zygosity: monozygotic (MZ) male (17), MZ female (22), dyzigotic (DZ) male (17), DZ female (18), DZ opposite sex (16). In two families, the twin pairs were incomplete. Twins were 16 or 17 years old at the day of testing (mean age 16.85, SD=0.36). Mean age of the siblings was 19.26 (SD=1.30).

**Table 2.1** – Number of participants included in the neuropsychological assessment and MRI scan on 1, 2 or 3 occasions.

| Assessment | Neuropsychological testing | MRI scan |
|---|---|---|
| 1+2+3 | 212 | 154 |
| 1+2 | 47 | 37 |
| 1+3 | 31 | 73 |
| 2+3 | 2 | 7 |
| Only 1 | 37 | 37 |
| Only 2 | - | 1 |
| Only 3 | 1 | 7 |
| Total | 330 | 316 |

## PROCEDURES

### *Invitation*

All participants and their parents were sent an invitation letter including a brochure (Appendices 1-3). Besides general information about the study purpose and procedure, it stated that participants would receive gift vouchers and compensation for travel expenses. Participants were asked to come to the UMC Utrecht for a six-hour visit. Further, as a token of appreciations, a summary of their results on a computerized tests (Appendix 4) and a printed image of their brain from the MRI scan would be mailed to them after the test day.

Following the invitation letter, participants were contacted by phone to inquire whether they had received the letter and were willing to participate. A telephone protocol was used that specified for each moment of contact: the date and time, what was discussed, whether each participant of a family was willing to participate, when to call back (if necessary), the reason for not participating (if applicable), the confirmed date of the appointment, and the date of sending the confirmation letter including study materials.

The twins and could participate together or make separate appointments if this was preferred, for example because of different school or work schedules. In addition, participants could choose to start in the morning or the afternoon, and could take part in the study on weekdays as well during the weekend.

When families agreed to participate, they were sent a confirmation of the appointment (Appendices 5-6) and consent forms (Appendices 7-8). This confirmation letter further included additional materials (for collection of urine, saliva and cheek swabs) and documents (instructions for hormone and buccal collection, MRI checklist, questionnaires, directions to the hospital; see Appendices 9-13).

### *Experimental procedures*

The data collection took place in the UMCU, similar to the second assessment. At the first assessment, the cognitive test protocol took place at the VU University Amsterdam and the MRI scan and physical examinations were made at the UMCU. For a family of 3 children, the test day lasted approximately 6 hours (including lunch; test protocol is described in Table 2.2). Depending on the availability of the MRI scanner and the preference of the participants,

the protocol for a family of 3 could be as follows: 1) morning neuropsychological tests, afternoon MRI scans (9:00-15:00); or 2) afternoon MRI scans, then neuropsychological tests (13:00-19:00); or 3) afternoon neuropsychological tests then MRI scans (14:00-20:00). If participants came alone or with two persons the protocol lasted 4 or 5 hours.



**Table 2.2** – Test protocol for the third measurement.

| | |
|---|---|
| *Welcome at the UMC Utrecht* | |
| Collection of questionnaires, saliva and urine samples, cheek swabs | 15 minutes |
| Explain procedure and sign consent forms | 15 minutes |
| *Neuropsychological test protocol* | |
| WAIS-III Intelligence test | 45 minutes |
| Break | 15 minutes |
| Computerized neuropsychological test battery (+ reading test) | 75 minutes |
| Lunch break | 45 minutes |
| *Afternoon program* | |
| MRI scan | 50 minutes |
| Corsi task | 5 minutes |
| Iowa Gambling task | 10 minutes |
| One minute reading test | 2 minutes |
| Physical examination (length, weight, blood pressure, Tanner stage) | 10 minutes |

The order of the afternoon program was different for each participant because of MRI scanner availability, and could vary depending on the length of the neuropsychological test assessment.

***Changes and continuity in data collection***

Compared to the data collection on the first two assessments, most conditions and instruments have stayed the same on the third assessment, to the extent that this was possible. MRI scanners and protocols did not change, physical assessments were the same and participants collected urine and saliva at home in the same way on two consecutive days. However, the cognitive assessment was adapted, so that psychometric IQ scores were now assessed by the test for adults and the collection of neurocognitive tests was replaced by an extensive computerized cognitive test battery. Details of the data collection of the third assessment will be described below. The main outcome variables for cognition, health and lifestyle collected at the third assessment are listed in Table 2.3, including the mean and SD separately for twins and siblings. Basic outcome variables of the MRI scans of all three assessments are listed in Table 2.4.

***Neuropsychological assessment***

*Intelligence*

A selection of subtests of the Wechsler Adult Intelligence Scale – Third version (WAIS-III Dutch version, Wechsler, 2004) was administered (in order of administration: Vocabulary, Block Design, Similarities and Matrix Reasoning). Raw test scores were standardized based on the age of the participant. Then a correction for the number of excluded subtests was

performed to be able to calculate IQ scores: the sum of the standardized score of Vocabulary and Similarities was multiplied by 2.5 (verbal IQ), the sum of standardized scores of Block Design and Matrix Reasoning was multiplied by 3 (performance IQ), the sum of verbal and performance IQ (total IQ). On the first assessment, the entire Wechsler Intelligence Scale for Children – Third Version (WISC-III Dutch version, Wechsler, 2002) was administered. On the second assessment, 6 subtasks of the WISC were administered. Standardization of the raw WISC scores was based on norm tables from 2003 (Nederlands Instituut van Psychologen Dienstencentrum, 2003).

*The Computerized Neurocognitive Battery*

The DCNB (Dutch Computerized Neurocognitive Battery) is a direct translation of the current webbased CNB (Gur et al., 2012). It includes a total of 17 tests, resulting in measures of performance accuracy (the percentage or number of correct responses) and reaction time in 5 overall cognitive domains. These domains, their corresponding test and which cognitive skill they specifically measure are given in Table 2.5. For more detailed descriptions of these tests we refer to (Gur et al., 2010; Gur et al., 2012). Prior to the administration of each of the DCNB's tests, instructions were read out loud to the participant by the administrator, after which participants were provided with practice trials (memory tasks and the conditional exclusion test excluded). These trials had to be completed successfully before the actual trials started. The administrators kept track of whether participant's test scores were valid or not, for example on the basis of the participant's (lack of) motivation, the presence of distracters, or computer issues. On top of this, automated test score validation occurred upon upload to the Pennsylvania web servers that host the CNB. Test duration was approximately 75 minutes.

*Iowa gambling task*

The Iowa gambling task measures decision making and self-regulation in a delayed reward task where the participant has to make decisions that may be more advantageous on either the short-term or the long-term (Bechara et al., 1994). Performance on this test is related to the damage to the frontal cortex, addiction, and risk taking behavior in adolescence (Brevers et al., 2013; Crone and van der Molen, 2004). Four decks of cards were presented on the computer screen, each with a different reward schedule. Participants had to click on a deck in order to receive either an award (most often) or a penalty. The magnitude of the reward and the penalty depended on the deck. The deck that has high reward on the short-term will also include high penalty, whereas the deck with low reward on the short/term includes less penalty, making this more advantageous on the long/term. The task started with a fictional $2000 and the participants were instructed to choose decks and try to earn as much money as they could. The task finished after 100 cards. The outcome was the total amount of money at the end of the task.

*Reading ability*

The participants were instructed to read out loud, within one minute, as many words as possible from a card with 116 words. The list was adapted from the "Three Minutes Reading Task", which is frequently used in the Dutch educational system (Cito, 1995).

**Table 2.3** – Overview of all main output variables collected at the 3th assessment. Means and SD are given separately for twins and siblings.

| Task or measure used | Main output phenotype | N total (twins / siblings) | mean ± sd twins | mean ± sd siblings | N total 1st | N total 2nd |
|---|---|---|---|---|---|---|
| Cognition | | | | | | |
| Intelligence (WAIS) | Total IQ | 176 / 70 | 100.4 ± 13.1 | 107.5 ± 14.1 | 224 / 102 | 178 / 81 |
| | Verbal IQ | 176 / 70 | 105.3 ± 13.6 | 108.2 ± 15.6 | 224 / 102 | 178 / 81 |
| | Performance IQ | 176 / 70 | 102.1 ± 12.0 | 108.0 ± 13.7 | 224 / 102 | 178 / 81 |
| Reading ability (1 minute) | Total correct words | 176 / 69 | 91.2 ± 14.8 | 95.8 ± 14.5 | 209 / 85 | 167 / 76 |
| Spatial memory (Corsi) | Total correct words | 176 / 70 | 9.2 ± 1.6 | 9.2 ± 1.7 | 221 / 101 | 173 / 79 |
| Decision making (Iowa gambling) | Total gain across items | 176 / 70 | 1678.1 ± 619.7 | 1858.6 ± 748.1 | - | - |
| CNB, cognitive domain | | | | | | |
| Attention | True positive responses (#) | 176 / 70 | 54.4 ± 4.9 | 55.9 ± 4.5 | - | - |
| | Median RT (ms) | 176 / 70 | 495.7 ± 43.2 | 486.4 ± 38.2 | - | - |
| Abstraction / mental flexibility | Correct categories (#) | 176 / 70 | 2.1 ± 0.6 | 2.1 ± 0.6 | - | - |
| | Median RT (ms) | 176 / 70 | 2359.2 ± 882.5 | 2471.7 ± 757.3 | - | - |
| Working memory | True positive responses (#) | 175 / 70 | 19.0 ± 1.7 | 19.1 ± 1.1 | - | - |
| | Median RT (ms) | 175 / 70 | 519.0 ± 107.7 | 524.4 ± 84.0 | - | - |
| Verbal memory | Total correct (#) | 176 / 70 | 36.7 ± 2.5 | 36.7 ± 2.6 | - | - |
| | Median RT (ms) | 176 / 70 | 1422.0 ± 199.5 | 1376.7 ± 198.0 | - | - |
| Verbal memory - Delayed | Total correct (#) | 176 / 70 | 36.0 ± 2.9 | 35.8 ± 2.9 | - | - |
| | Median RT (ms) | 176 / 70 | 1402.5 ± 232.1 | 1355.0 ± 217.2 | - | - |
| Face Memory | Total correct (#) | 176 / 70 | 31.2 ± 3.5 | 31.8 ± 3.3 | - | - |
| | Median RT (ms) | 176 / 70 | 1959.0 ± 490.2 | 1945.4 ± 491.7 | - | - |
| Face Memory -Delayed | Total correct (#) | 176 / 70 | 32.7 ± 3.5 | 32.4 ± 3.4 | - | - |
| | Median RT (ms) | 176 / 70 | 1730.4 ± 425.1 | 1691.4 ± 325.7 | - | - |
| Spatial memory | Total correct (#) | 176 / 70 | 16.4 ± 2.2 | 16.5 ± 2.1 | - | - |
| | Median RT (ms) | 176 / 70 | 1696.9 ± 401.9 | 1707.1 ± 366.1 | - | - |
| Spatial memory - delayed | Total correct (#) | 176 / 70 | 15.8 ± 2.3 | 16.0 ± 2.7 | - | - |
| | Median RT (ms) | 176 / 70 | 1538.7 ± 353.5 | 1562.5 ± 297.5 | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Nonverbal reasoning | | Total correct (#) | 176 / 70 | 16.4 ± 4.0 | 17.7 ± 3.9 | - | - |
| | | Median RT (ms) | 176 / 70 | 10195.2 ± 5135.5 | 11229.4 ± 5844.9 | - | - |
| Language reasoning | | Percentage correct | 176 / 70 | 65.4 ± 18.7 | 68.6 ± 19.5 | - | - |
| | | Median RT (ms) | 176 / 70 | 8739.5 ± 3325.0 | 8513.8 ± 3271.0 | - | - |
| Spatial ability | | Total correct (#) | 176 / 70 | 13.6 ± 3.5 | 14.3 ± 3.7 | - | - |
| | | Median RT (ms) | 176 / 70 | 9624.9 ± 2828.7 | 10177.5 ± 2443.2 | - | - |
| Emotion Identification | | Total correct (#) | 176 / 70 | 33.6 ± 2.9 | 33.8 ± 2.8 | - | - |
| | | Median RT (ms) | 176 / 70 | 1962.0 ± 368.7 | 2020.6 ± | - | - |
| Emotion Differentiation | | Total correct (#) | 176 / 70 | 28.7 ± 3.2 | 29.5 ± 2.7 | - | - |
| | | Median RT (ms) | 176 / 70 | 3164.2 ± 882.1 | 3266.9 ± 814.6 | - | - |
| Age Differentiation | | Total correct (#) | 176 / 70 | 27.5 ± 3.3 | 28.1 ± 3.0 | - | - |
| | | Median RT (ms) | 176 / 70 | 2546.5 ± 842.1 | 2626.9 ± 813.4 | - | - |
| Sensorimotor speed | | Total correct (#) | 176 / 70 | 20.0 ± 0.0 | 20.0 ± 0.0 | - | - |
| | | Median RT (ms) | 176 / 70 | 472.5 ± 1044.0 | 680.6 ± 102.6 | - | - |
| Motor speed | | Total taps in 1 minute | 176 / 69 | 38.7 ± 78.3 | 59.6 ± 7.0 | - | - |
| Physical examination | | | | | | | |
| Height | | Centimeters | 176 / 70 | 173.6 ± 8.2 | 175.1 ± 9.5 | 218 / 99 | 174 / 78 |
| Weight | | Kilogram | 176 / 70 | 64.1 ± 9.5 | 70.1 ± 10.6 | 218 / 99 | 174 / 78 |
| Diastolic blood pressure | | mmHG | 176 / 70 | 71.0 ± 9.4 | 72.7 ± 9.6 | - | - |
| Systolic blood pressure | | mmHG | 176 / 70 | 129.1 ± 13.4 | 133.6 ± 14.4 | - | - |
| Heart rate | | Beats per minute | 176 / 70 | 64.7 ± 11.6 | 65.1 ± 12.0 | - | - |
| Smoking | | Yes / no / stopped (%) | 133 / 54 | 23/ 71/ 6 | 26/ 70/ 4 | - | - |
| Puberty | | | | | | | |
| Tanner stage | Boys | Penis development (1:5) | 77 / 26 | 1/ 2/ 13/42/19 | 0/ 0/ 3/ 11/ 12 | 108 / 44 | 83 / 31 |
| | | Pubic hair (1:6) | 78 / 26 | 1/ 0/ 1/ 1/ 46 / 13 | 0/ 0/ 0/ 3/ 15/ 8 | 107 / 44 | 84 / 31 |
| | | Testis size size (1:4) | 78 / 26 | 0/ 10/ 34/ 34 | 0/ 2/ 9/ 15 | - | - |
| | Girls | Breast development (1:5) | 96 / 44 | 0/ 0/ 0/ 40/ 56 | 0/ 0/ 0/ 6/ 38 | 109 / 54 | 86 / 43 |
| | | Pubic hair (1:6) | 96 / 44 | 4/4/1/13/55 /19 | 0/ 0/ 1/ 5/ 20/ 18 | 108 / 53 | 80 / 44 |

**Table 2.3** – Continued.

| Task or measure used | Main output phenotype | N total (twins / siblings) | mean ± sd twins | mean ± sd siblings | N total 1st | N total 2nd |
|---|---|---|---|---|---|---|
| Hormones | | | | | | |
| Morning urine | | 166 / 62 | | | 223 / 101 | 177 / 77 |
| Saliva | | 168 / 63 | | | 215 / 99 | 175 / 79 |
| Testosterone - Boys | pmol/liter | 80 / 25 | 344.2 ± 128.8 | 390.0 ± 131.0 | 106 / 41 | 86 / 37 |
| Testosterone – Girls | pmol/liter | 88 / 38 | 56.5 ± 51.8 | 71.86 ± 78.0 | 108 / 45 | 88 / 43 |
| Questionnaires | | | | | | |
| Child behavior checklist (CBCL) | Report by parents | 174 / 69 | | | 158 / 92 | 198 / 81 |
| Dutch health behavior questionnaire (DHBQ) | Report by participant | 173 / 68 | | | - | 175 / 81 |
| Adult self rating (ASR) | Report by parents | 80 fathers, 88 mothers | | | - | - |

Note: testes volume is mean left & right as measured with orchidometer; pubic hair is measured with a 6-point scale, however, both stage 5 and 6 are adult like appearance.

**Table 2.4** – Main output variables (mean ± SD) of MRI scans at each of the three assessments.

| | Assessment 1 | Assessment 2 | Assessment 3 |
|---|---|---|---|
| Total brain volume (ml) | 1329 ± 111 | 1340 ±120 | 1323 ± 128 |
| Total gray matter (ml) | 813 ± 67 | 796 ± 71 | 742 ± 74 |
| Total white matter (ml) | 516 ± 56 | 544 ± 61 | 581 ± 69 |
| White matter FA | 0.44 ± 0.2 | 0.45 ± 0.02 | 0.43 ± 0.03 |

Total number of participants with a good T1 scan at assessment 1 was 275; 184 at assessment 2, and 233 at assessment 3. Good quality DTI scans (for FA) was present for 264, 169, 228 participants at assessment 1, 2 and 3 respectively.



**Table 2.5** – Overview of the global cognitive functions, corresponding tests and the cognitive domain they measure

| Cognitive Function | Test name | Cognitive domain measured |
|---|---|---|
| *Executive-control* | Continuous Performance Test | attention |
| | Letter N-Back Test | working memory |
| | Conditional Exclusion Test | abstraction and mental flexibility |
| *Episodic memory* | Face Memory Test | face memory |
| | Word Memory Test | verbal memory |
| | Visual Object Learning Test | spatial memory |
| *Complex cognition* | Matrix Reasoning Test | nonverbal reasoning |
| | Verbal Reasoning Test | language reasoning |
| | Line Orientation Test | spatial ability |
| *Social cognition* | Emotion Identification Test | emotion identification |
| | Emotion Differentiation Test | emotion differentiation |
| | Age Differentiation Test | age differentiation |
| *Sensorimotor speed* | Motor Praxis Task | sensorimotor speed |
| | Finger Tapping Test | motor speed |

### *Behavioral data and lifestyle information*

*Questionnaires*

Twins, siblings and their parents, were asked to fill in standardized questionnaires. Participants filled in the Dutch Health and Behavior Questionnaire, which includes questions on emotional and behavior problems (Youth Self-Report; Achenbach, 1991), well-being, lifestyle, exercise behavior, sedentary behavior, education, and family functioning (van Beijsterveldt et al., 2013). Parents were asked to fill in the Adult Self-Report (ASR; Achenbach and Rescorla, 2003) about themselves (fathers 80, mothers 88), and the Child Behavior Checklist (CBCL; Achenbach and Rescorla, 2001) about their children. We received 241 surveys from the participants and 243 from their parents.

*Education*
Participants were asked about their own educational background, as well as that of their parents. Level of education was defined as the sum of years involved in elementary, secondary and higher education if the educational curriculum (per year) was completed.

*Medication*
Participants were asked to bring with them any packages of medication they used at the moment of testing, or very recently had used. Medication use was rare and complete information was not always provided by the participants. Concerning use of contraceptive pills, girls were asked to mention the brand of the pill on the questionnaire about urine collection (see Appendix 13).

*Smoking behavior*
At the start of the testing day, participants were individually (in private) asked about their current or previous smoking behavior. When they mentioned they had smoked or were still smoking, the researcher asked for the exact age of initiation (as close as possible), frequency of smoking and the age of quitting.

***Physical examination and DNA and hormone sample collection***

*Length and weight*
Participants were asked to take off their shoes before body height and weight were measured.

*Blood pressure*
Blood pressure and heart rate were measured in a sitting position with an Omron automatic blood pressure monitoring device. The cuff was attached to the non-dominant arm. Participants were asked to remain relaxed and still and refrain from talking and laughing.

*Tanner stage*
Tanner stages were determined with a self-report questionnaire, on the basis of secondary sexual characteristics using the five stages of development devised by Marshall & Tanner (Marshall and Tanner, 1969; Marshall and Tanner, 1970). After explanation by the researcher, the researcher left the room and participants were asked to fill in their developmental status on black and white photographs of the different pubertal stages. Stage 1 represents no pubertal development and full maturation is represented at stage 5. Girls were asked about breast development and pubic hair growth; boys were asked about genital development and pubic hair growth. In boys, genital stage was divided in penis and testes development. Testes volume was reported on a 4-item scale (compared size with ovals: 1) 1-3 ml; 2) 4-6 ml; 3) 7-11ml; 4) 12-25 ml) and boys were also asked to rate testes volume with an orchidometer.

*Collection of urine and saliva*
As at the previous occasions, participants were asked to collect saliva and morning urine for the assessment of reproductive hormonal levels (LH, FSH, estrogen, and testosterone (in saliva)) on two consecutive days. Because of their hormonal cycle, girls were asked to collect urine and saliva for assessment of hormone levels at a specific time point during their menstrual cycle, namely in the early follicular phase when hormone levels are relatively low. To

minimize effects of contraceptive pills, they were asked to collect morning urine and saliva at the 6th and 7th day of their menstrual cycle. They could send the samples by mail to the VU University. Boys were asked to collect samples in the two days prior to the test day and bring the samples to the UMC Utrecht. They could also send the samples by mail.

Participants were asked to fill in the time and date of the collection. Girls had a more extensive questionnaire that included questions about their menstrual cycle (see Appendices 12-13).

Samples were stored in the refrigerator as soon as possible. After pipetting a small volume of urine for hormone level assessment, all samples were stored at -20˚C at either the VU (when samples came by mail) or at UMCU (when participants brought urine samples with them at test day). Saliva samples were stored at -20˚C as soon as possible. When applicable, samples were brought over from UMCU to VU as soon as possible.

*Assessment of hormone levels*

Determination of hormone levels at all three assessments was carried out by the endocrine laboratory of clinical chemistry of the VU Medical Center in Amsterdam, the Netherlands. For the first two assessments, LH, FSH and estradiol levels were determined in morning urine using highly sensitive immunometric assays (Luminiscention) (Architect, Abbott Laboratories, Abbott Park, IL, USA). The detection limits of LH and FSH were 0.1 U/L and 0.11 U/L respectively, with an intra-assay coefficient of variation (CV) of 3% and inter-assay CV of 6%. For estradiol, the intra-assay and inter-assay CVs were 5% and 10% respectively at levels >150 pmol/L (lower) and <9000 pmol/L (upper). Creatinin concentrations (to control for variance in urine excretion rate) were measured by the Jaffé method (Modular, Roche Diagnostics, Mannheim, Germany). Inter-assay coefficient of variation is 2.2% at 5.9 mmol/L and 1.7% at 12.5 mmol/L. Free testosterone levels were determined in first morning saliva (Competitive immunoassay (luminiscention), IBL Hamburg). The intra-assay and inter-assay CVs were below 12% at levels >11 pmol/L (lower limit of detection). For the third assessment, testosterone levels were determined via isotope-dilution liquid chroma-tography–tandem mass spectrometry. Intra-assay coefficient of variation (CV) was 11%, 4%, and 2% at 10, 140, and 900 pmol/L, respectively. Inter-assay CV was 5% at 200 and 2000 pmol/L, respectively. See Büttler et al. (2016) for more details. Despite the different methods, the results of the different assessments are comparable; the only difference is that the new method is more reliable with less variance (Büttler et al., 2016; AC Heijboer, endocrine laboratory VUmc, personal communication).

*Buccal epithelium*

Participants were asked to collect buccal swabs in the morning and evening, on 2 days. They were instructed not to eat, drink or brush their teeth prior to collection. Collection of buccal epithelium was done by rubbing cotton buds along the inside of the mouth. The swabs were then placed in a tube with buffer. Participants could bring the tubes with them to the UMCU at test day, or send the rubes by postal mail. DNA was isolated at the *Avera Institute for Human Genetics,* Sioux Falls. All samples were tested on single nucleotide polymorphic (SNP) markers to establish zygosity (van Beijsterveldt et al., 2013)

***MRI scan protocol***

At the third assessment, the same scan parameters were used as in the previous two test assessments (see Table 2.6) where participants were scanned at a 1.5 Tesla Philips Achieva scanner (Brouwer et al., 2012; Peper et al., 2008; van Soelen et al., 2012a). At the start of the test day, the scan procedure was explained to the participants. Presence of wires (top/ bottom/ both) was asked as this may distort the MRI image; it was not an exclusion criterion for MRI scanning. They were allowed to watch a movie or listen to music during the structural scans. During the last 10 minutes the resting state functional MRI scan was made, for which participants were asked to close their eyes and try to think of nothing specific. Afterwards, participants were asked if they had remained awake during the scan and if they managed to think of nothing specific. The total scan protocol took about 45-50 minutes per child.

**Table 2.6** – Scan protocol and MR acquisition details.

| | MR acquisition details | Duration |
|---|---|---|
| 1. | Scout scan, sagittal T1 weighted; TR = 13 ms; TE = 4.6 ms | 1 min. |
| 2. | Dual Echo – Turbo Spin Echo (DE-TSE) clinical scan, transversal T2 weighted; TR=2200 ms, TE= 9ms; 19 slices of 6 mm; slicegap 1 mm; flipangle 90°; FOV: 230 mm / 90% | 2 min. |
| 3. | Three Dimensional - Fast Field Echo (3D-FFE) T1 weighted scan; coronal; 256 x 212 acquisition matrix; 256 × 256 reconstruction matrix; 160–180 contiguous slices of 1.2 mm; TR = 30 ms; TE = 4.6 ms; flipangle 30°; FOV: 256 mm / 65% | 7 min. |
| 4. | 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 x 96 acquisition matrix; reconstruction matrix 128 x 128; flipangle 90°; FOV: 240 mm; TE = 60–88 ms; no cardiac gating. | 11 min. |
| 5. | Magnetization Transfer Imaging (MTI) scan; 60 transverse slices of 2.5 mm; slicegap 0.0 mm; 128 x 96 acquisition matrix; reconstruction matrix 128 x 128; flip angle 8°; FOV: 240 mm / 78%; TR = 37.5 ms; TE = 3.73 ms. | 7 min. |
| 6. | Dual Echo - Turbo Spin Echo using SENSE, transversal T2 weighted; parallel imaging, sensefactor 2; TR/TE1/TE2 6000/18/80 ms; 120 slices of 2 mm; 256 x 195 acquisition matrix; reconstruction matrix 256 x 256; slicegap 0 mm; flipangle 90° ; FOV: 240 mm / 79 % | 7 min. |
| 7. | Resting State Scan using SENSE coil; parallel imaging, sensefactor 1.8; 3D T2* weighted field echo EPI (FEEPI) scan; Timeseries 800-1200 scans, single scan duration 0.5-0.7 sec; sagittal scanorientation; acquisition matrix: 64 x 33; reconstruction matrix: 64 x 64; flip angle 9°; 36 slices; FOV: 256 mm; 4 mm isotropic voxels; TR=21.1 ms; TE= 31.10 (shifted echo). | 10 min. |

At first assessment, T2 (scan 6) and rs-fMRI (scan 7) were not included; all other scan parameters were the same

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Chapter 3

# Longitudinal study of hormonal and physical development in young twins

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**ABSTRACT**
Information on the correlation of normative reproductive hormone levels with physical development (Tanner stages) during puberty and on the influences of genes and environment on variation in these hormones and Tanner stages is limited. One hundred twelve healthy 9-year-old twin pairs (n = 224) took part in this longitudinal study, of which 89 pairs participated again at age 12 years (n = 178). Morning urinary LH, FSH, estradiol, and salivary testosterone levels, determined by competitive immunoassays, were measured. Tanner stages were determined through physical examination. Over the 3-year interval, all hormone levels showed a 2- to 9-fold increase. LH and FSH at age 9 years predicted sex-specific Tanner stages at age 12 years in both boys and girls. Most of the associations between hormone levels at age 9 years and physical development at 12 years were explained by genetic influences. FSH in 9-year-old boys correlated with all hormone levels and Tanner stages at age 12 years. Moderate to high heritability estimates were found for hormone levels at both ages and in both sexes. In girls a shift from environmental (age 9 years) to genetic influences (age 12 years) was found for estradiol and pubic hair development, and for breast development a shift in the opposite direction was seen. In conclusion, during development LH and FSH (and testosterone in boys) levels predict secondary sexual characteristics in boys and girls 3 years later. These correlations are largely due to genes that are involved in both early pubertal hormone levels and subsequent physical development.



**INTRODUCTION**
Puberty is characterized by the development of secondary sexual characteristics and physical growth. The general hypothesis on the endocrine onset of puberty is thought to proceed in a series of steps that concern the reactivation of the hypothalamus-pituitary-gonadal (HPG) axis (see, for example, Cameron, 2004; Delemarre-Van De Waal, 2002; Grumbach, 2002; Shirtcliff et al., 2009): first, the pituitary is stimulated by the hypothalamus (via GnRH neurons) to secrete nightly LH and FSH surges 1–2 years before the physical onset of puberty. After some time, LH and FSH pulses also occur during the day. These hormones in turn stimulate the gonads (the testis or ovaries) to produce estrogen and testosterone, which play an important role in the onset of physical (secondary sexual characteristics) and behavioral changes.

Reproductive hormones regulate important changes during puberty. Knowledge on relative influences of genes and environment on individual differences in these hormone levels will provide relevant information on the (endocrine) regulation of puberty. Twin studies are widely used in various fields (see, for example Ring et al., 2005 for assessment of genetic influences on sex hormones in adult males) to estimate the extent to which variation in a certain trait is caused by genetic and/or environmental factors. Monozygotic (MZ) twins are genetically identical and share (nearly) 100% of their genetic material, whereas dizygotic (DZ) twins share, like siblings, on average 50% of their segregating genes. If for a certain variable MZ twins are more alike than DZ twins, it can be inferred that the variable is influenced by genetic factors (see Boomsma et al., 2002 for an overview of twin research, and see Falconer and Mackay, 1992; Neale and Cardon, 1992; Posthuma et al., 2003 for a more detailed description). There are some studies that report on the heritability of reproductive hormones in prepubertal boys [5–11 years old (Wang et al., 2004)], and testosterone

in teenagers [14–21 years old (Harris et al., 1998); 12 years old (Hoekstra et al., 2006)]. These studies found moderate to strong contributions of genetic effects on the variation of reproductive hormone levels. However, to our knowledge, the longitudinal aspect of genetic influences on hormone levels during this critical period of development has not been investigated.

In the current study, the development of HPG axis hormones during puberty is described in a longitudinal twin study (n = 224 individuals). LH, FSH, estradiol, testosterone, and secondary sexual characteristics were measured at 9 (baseline) and 12 (follow-up) years of age. The twin design allowed an estimate of the extent to which variation in reproductive hormones, and the extent to which correlations among hormones and physical development are driven by genes during puberty.



## MATERIALS AND METHODS

### *Participants*

Healthy twins (224 individuals) were recruited from The Netherlands Twin Register (Boomsma et al., 2006). They took part at age 9 and 12 years (n = 178 individuals) in an ongoing longitudinal study on the development of cognition and brain structure and function (van Soelen et al., 2012) (Table 3.1). The Central Committee on Research involving Human Subjects of The Netherlands approved the study. Written informed consent was obtained from parents and children.

### *Analysis of hormone levels*

Participants were asked to collect first morning urine and saliva immediately after waking up on 2 consecutive weekdays. Analyses were carried out by the endocrinological laboratory of clinical chemistry of the VU University Medical Center (Amsterdam, The Netherlands).

Determination of urinary LH, FSH, and estradiol, and salivary testosterone have been described elsewhere (Peper et al., 2010). Urinary LH, FSH, and estradiol levels were divided by creatinine level to correct for variations in urine excretion rate. A creatinine correction has been demonstrated to enhance the detection of LH surges (Kesner et al., 1998). Creatinine concentrations were measured by the Jaffé method (Modular; Roche Diagnostics, Mannheim, Germany). The interassay coefficient of variation was 2.2% at 5.9 mmol/L and 1.7% at 12.5 mmol/L.

### *Assessment of Tanner stages*

Secondary sexual characteristics of puberty were measured by a trained researcher at the University Medical Center Utrecht (Utrecht, The Netherlands) on the basis of the characteristics of sexual development devised by Marshall and Tanner (Marshall and Tanner, 1969; Marshall and Tanner, 1970). In boys, genital stage was divided in penis and testes development. Testes volume was reported on a 4-item scale; at age 12 years, testes volume was measured with an orchidometer. When children felt uncomfortable with the assessment, they were asked to point out their status on black and white photographs of the different puberty stages accompanied by an explanation by the researcher (only at age 12 years; 16 girls, 28 boys).

***Statistical analyses***

Differences in hormone levels over time and between boys and girls were tested using structural equation modeling with the software package OpenMx (Boker et al., 2011) to control for the dependency of the data.

Hormone levels assessed at 2 days, available for more than 90% of the participants, were averaged. A log transformation was applied to normalize the data. Many of the 9-year-old children had LH levels that were below the detection limit (58 girls, 50 boys), but these low levels still provide information. These values were included in the genetic analysis by setting all values that were below the detection limit to a half of the detection limit. To check whether this procedure influenced the results, all genetic analyses were repeated with undetectable levels left out. Dissimilar results are reported in footnotes. After the log transformation, all data were normally distributed, except LH in 9-year-old girls (Kolmogorov-Smirnov $Z = 1.369$, $P = .047$).

***Genetic analysis***

Relative influences of genetic factors and environmental factors can be examined by comparing within-pair correlations between MZ and DZ twins. When an MZ correlation is twice as high as a DZ correlation, this indicates that a variable is largely influenced by genetic factors. The proportion of variance in a trait that can be attributed to genetic factors is termed heritability. In addition to genetic factors, resemblance between twins can arise from common environment, which comprises those environmental factors that induce similarity in children growing up in the same family. The presence of common environmental factors is suggested when correlations in DZ twins are larger than half the MZ correlation [as implemented in a full model that allows estimation of genetic (A), shared environmental (C), and nonshared environmental effects (E) (ie, ACE model) (Boomsma et al., 2002)]. When the MZ correlations are more than twice the DZ correlations, there is a suggestion for nonadditive genetic influences [epistasis or dominance, implemented in a model that allows estimation of additive genetic effects (A), nonadditive genetic effects (D), and non-shared environmental effects (E) (i.e., ADE model) (Falconer and Mackay, 1992)]. Unique environmental influences are not shared with other family members and also contain the measurement error (Falconer and Mackay, 1992). The proportion of the total variance that can be attributed to genetic or environmental factors gives estimates of (univariate) heritability ($h^2$), unique environmental influence, and common environmental influence (in the case of an ACE model), or nonadditive genetic influences ($d^2$) (in the case of an ADE model). In the latter case, we present estimates of broad heritability ($h^2 + d^2$).

Ordinal data, such as Tanner stages, were analyzed under the assumption that there is an underlying continuous liability with mean 0 and variance 1 to the observations. Four thresholds divide the liability into the 5 Tanner stages (4 for testes development); hence, 4 thresholds (3 for testes development) were estimated. Estimates of thresholds are based on the prevalence of the Tanner stages in the sample (Neale and Cardon, 1992).

To increase power, heritability was estimated from bivariate models (Neale and Cardon, 1992) in which variables assessed at age 9 and 12 years were analyzed simultaneously. The phenotypic correlation (Rph) among continuous traits (hormone levels), between ordinal Tanner stages, and among Tanner stages and hormone levels, were estimated in Open-

**Table 3.1** – Normative hormone levels and Tanner stages in 9- and 12-year old boys and girls

| **Boys**[a] | | | | | |
|---|---|---|---|---|---|
| N (MZ/ DZ/ DOS individuals) | Age 9 yr<br>46/ 44/ 20 | | Age 12 yr<br>40/ 34/ 15 | | |
| | Mean (sd) | N | Mean (sd)[b] | N | Increase (x-fold) |
| Age | 9.10 (0.10) | 110 | 12.13 (0.24) | 89 | 3.04 (0.23)[c] |
| BMI (kg/m2)[d] | 16.21 (1.36) | 103 | 18.54 (1.99) | 87 | |
| LH (U/ mmol creat) | 0.02 (0.04) | 59[e] | 0.17 (0.14) | 89 | 6.9 |
| FSH (U/ mmol creat) | 0.25 (0.16) | 110 | 0.44 (0.26) | 89 | 1.8 |
| E2 (pmol/ mmol creat) | 126.55 (121.58) | 108 | 205.61 (138.54) | 89 | 1.6 |
| T (pmol/ L) | 24.98 (25.32) | 106 | 74.23 (86.54) | 86 | 3.0 |
| Tanner | N per stage | N | N per stage | N | |
| Tanner-P 1/2/3/4/5 | 100/ 5/ 1/ 1/ 0 | 107 | 20/ 37/ 21/ 5/ 0 | 83 | |
| Tanner-T 1/2/3/4 | 98/ 8/ 1/ 0 | 107 | 16/ 37/ 12/ 3 | 68 | |
| Tanner-PH 1/2/3/4/5 | 96/ 10/ 0/ 0/ 0 | 106 | 24/ 31/ 22/ 6/ 0 | 83 | |
| **Girls**[f] | | | | | |
| N (MZ/ DZ/ DOS individuals) | Age 9<br>50/ 42/ 20 | | Age 12<br>40/ 34/ 15 | | |
| | Mean (sd) | N | Mean (sd) | N | Increase (x-fold) |
| Age | 9.10 (0.09) | 112 | 12.17 (0.28) | 89 | 3.07 (0.26)[c] |
| BMI (kg/m2)[d] | 16.32 (1.99) | 106 | 18.18 (2.99) | 82 | |
| LH (U/ mmol creat) | 0.02 (0.03) | 53[g] | 0.21 (0.21) | 85 | 9.3 |
| FSH (U/ mmol creat) | 0.47 (0.27)[h] | 112 | 0.92 (0.47)[h] | 87 | 1.9 |
| E2 (pmol/ mmol creat) | 116.13 (77.96) | 112 | 366.77[h] (285.73)* | 87 | 3.2 |
| T (pmol/ L) | 31.04 (24.04)[h] | 108 | 58.00 (36.07) | 88 | 1.9 |
| Tanner | N per stage | N | N per stage | N | |
| Tanner-B 1/2/3/4/5 | 89/ 20/ 0/ 0/ 0 | 109 | 10/ 16/ 36/ 17/ 7 | 86 | |
| Tanner-PH 1/2/3/4/5 | 91/ 17/ 0/ 0/ 0 | 108 | 17/ 19/ 18/ 24/ 5 | 83 | |

Abbreviations: DOS, dizygotic opposite sex; E2, estradiol; T, testosterone. Values below detection limit were excluded from the descriptives, therefore, LH levels are an overestimation of actual levels in 9-year-old boys and girls.

[a] All hormone levels were increased at age 12 compared with age 9 ($P < 0.0001$).

[b] In total, 224 individuals participated in this study, with 46 DZ males at age 9. However, no hormone data was available for one DZ male twin pair, leading to a total N of 222.

[c] Mean (SD) duration between measurement 1 and measurement 2 (in years).

[d] Based on BMI tables for Dutch children described elsewhere (40), 14 girls (12.5%) and 4 boys (3.6%) at age 9 years were considered to be overweight, and 2 girls (1.8%) were considered to be underweight. At age 12 years, 15 girls (16.9%) and 9 boys (10.1%) were considered to be overweight, and 1 girl (1.2%) was underweight.

[e] N below detection limit = 50

[f] At follow-up, 14 girls (16%) had attained menarche, of which 3 reported to have regular cycles. None of the participants used oral contraceptives.

[g] N below detection limit = 58

[h] Significantly higher values in girls, see text.

Mx (Boker et al., 2011). Due to low variability in Tanner stages at age 9 years, we chose to compute the correlations of hormone levels at age 9 and 12 years with Tanner stages at age 12 years only. To estimate correlations among hormone levels and Tanner stages, hormone levels were normalized and categorized in five groups.

Significant phenotypic correlations were decomposed in a genetic (Rg) and (unique) environmental (Re and Rc) component (see Supplemental Figure 3.1). The magnitude of these underlying components is based on the comparison of cross-trait/cross-twin correlations for MZ and DZ twins. For example, if the cross-correlation between one hormone of twin A and another hormone of twin B is larger in MZ than in DZ twins, this indicates that a common genetic factor (partly) influences both hormones. The extent of the shared genetic influences is reflected by the magnitude of the genetic correlation. Rc and Re are interpreted likewise (Neale and Cardon, 1992).



Because the mechanism underlying hormone levels (and subsequent physical changes) may differ in boys and girls, we chose to present the results for boys and girls separately. Data from boys and girls from DZ opposite-sex pairs were included with the data from the male and female DZ twins and thus contribute to the estimation of mean and variances, but we did not include their covariance. See Supplemental Table 3.1 for twin correlations.

All data analyses were carried out with structural equation modeling in the software package OpenMx (Boker et al., 2011). All data were included in the analyses, regardless of 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.

## RESULTS

### *Normative hormone levels at ages 9 and 12 years*

For both boys and girls, all hormone levels were increased at age 12 years compared with age 9 years ($P < 0.0001$) (Table 3.1 and Figure 3.1; Supplemental Figures 3.2 and 3.3). The highest increase was seen for LH (~7-fold in boys, ~9-fold in girls). FSH levels increased about 2-fold in both boys and girls. Testosterone increased 3-fold in boys, approximately 2-fold in girls, whereas for estradiol this was the other way around (~2-fold increase in boys, ~3-fold in girls). At age 9 years, girls had higher levels of FSH ($P < 0.0001$) and testosterone ($P = .04$) than boys. At age 12 years, girls had higher levels of FSH ($P < 0.0001$) and estradiol ($P < 0.0001$) than boys.

At age 9 years, most of the children were in Tanner stage 1 [boys: 91%, 92%, and 93% for penis (Tanner-P), testes (Tanner-T), and pubic hair (Tanner-PH) development, respectively; girls: 82% and 84% for breast (Tanner-B) and Tanner-PH development], i.e., they had not yet started pubertal development. At age 12 years, most girls were in Tanner stage 3 (Tanner-B, 42%) or 4 (Tanner-PH, 29%) whereas most boys were in stage 2 (Tanner-P: 45%; Tanner-T: 54%; Tanner-PH: 37%). As can be seen in Table 1, at age 9 years, girls were only slightly further in their pubertal development than boys, whereas at age 12 years, girls were further developed than boys (based on Tanner stages).

***Genetic analyses of hormone levels and Tanner stages***

Significant genetic influences were found for variation in all hormones in boys and girls at both ages, except for estradiol at age 9 years in girls (Table 3.2; for unstandardized estimates see Supplemental Table 3.2). Overall, genetic influences were higher in boys compared with girls. Genetic influences did not change much over time, except for estradiol: in boys, the genetic influences seemed to become smaller over time. In girls, we found a significant influence of common environmental factors for estradiol at age 9 years. At age 12 years, these common environmental influences were no longer significant, and a significant proportion of the variance could be explained by genetic factors.

Over the 3-year interval, estimates of factors that influenced physical development changed. In boys, genetic influences for Tanner-T development were higher at age 12 years, whereas genetic influences for pubic hair development decreased over time. In girls, influences on Tanner-B development showed a shift over time: from genetic at age 9 years to



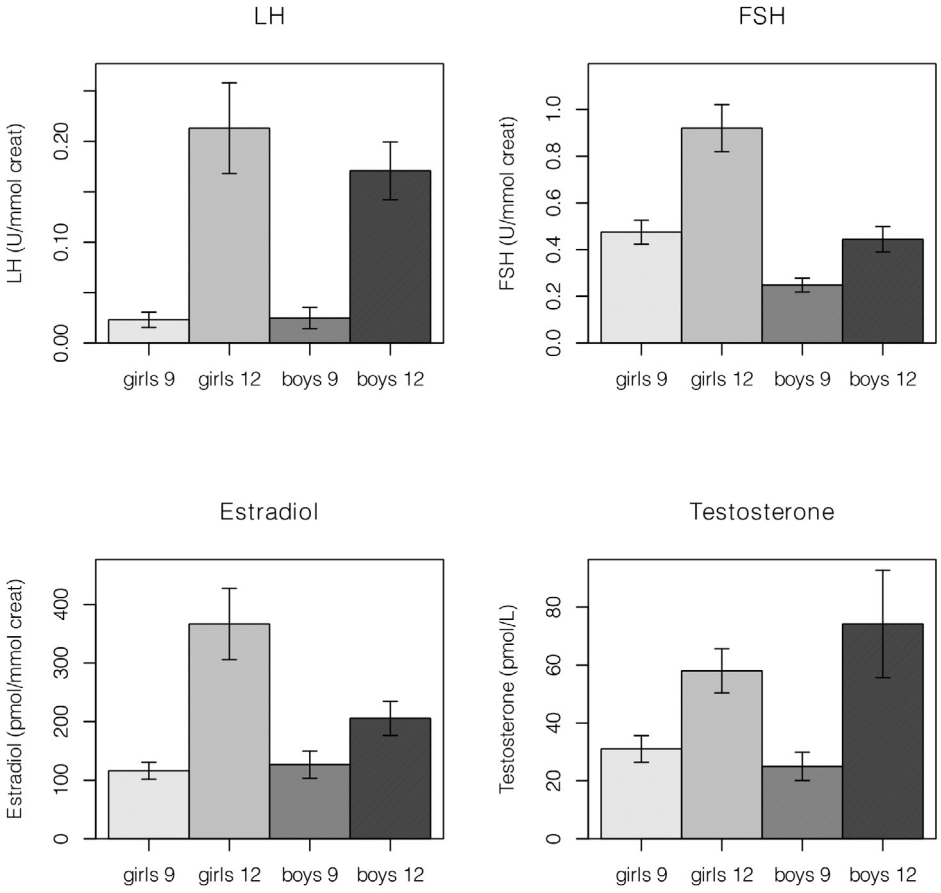


**Figure 3.1** - Normative hormone levels in 9- and 12-year-old boys and girls. LH, FSH, estradiol, and testosterone levels increased between age 9 and 12 years, in both boys and girls. At age 9 and 12 years, girls had higher FSH levels than boys; testosterone at age 9 years and estradiol at age 12 years were higher in girls compared with boys. Error bars represent 95% confidence interval.

common environmental influences at age 12 years. For Tanner-PH development in girls, environmental influences at age 9 years shifted to genetic influences at age 12 years.

**Table 3.2** – Influences of genes and environment on hormone levels and physical development of boys and girls during puberty.

| | Model[a] | (Broad) Heritability (95% CI) | Common Environment (95% CI) | Unique Environment (95% CI) |
|---|---|---|---|---|
| **Boys** | | | | |
| Age 9 | | | | |
| **LH** | ADE | **0.83** (0.66-0.90) | - | 0.17 (0.10-0.34) |
| **FSH** | ADE | **0.89** (0.78-0.94) | - | 0.11 (0.06-0.22) |
| **E2** | ACE | **0.72** (0.39-0.91) | 0.13 (0.00-0.45) | 0.14 (0.08-0.28) |
| **T** | ADE | **0.64** (0.30-0.81) | - | 0.36 (0.19-0.70) |
| **Tanner-P** | ACE | 0.09 (0.00-1.00) | 0.87 (0.00-1.00) | 0.04 (0.00-0.98) |
| **Tanner-T** | ACE | **0.40** (0.04-0.96) | 0.51 (0.00-0.91) | 0.09 (0.00-0.38) |
| **Tanner-PH** | ACE | **0.81** (0.07-0.99) | 0.07 (0.00-0.84) | 0.12 (0.00-0.97) |
| Age 12 | | | | |
| **LH** | ADE | **0.93** (0.85-0.96) | - | 0.07 (0.04-0.15) |
| **FSH** | ADE | **0.95** (0.89-0.97) | - | 0.05 (0.02-0.11) |
| **E2** | ACE | **0.45** (0.12-0.77) | 0.12 (0.00-0.40) | 0.43 (0.23-0.88) |
| **T** | ADE | **0.78** (0.59-0.88) | - | 0.22 (0.12-0.41) |
| **Tanner-P** | ACE | 0.09 (0.00-0.93) | 0.30 (0.00-0.66) | 0.62 (0.33-1.00) |
| **Tanner-T** | ACE | **0.72** (0.27-1.00) | 0.18 (0.00-0.47) | 0.10 (0.01-0.31) |
| **Tanner-PH** | ACE | 0.33 (0.00-0.90) | 0.49 (0.00-0.84) | 0.19 (0.07-0.43) |
| **Girls** | | | | |
| Age 9 | | | | |
| **LH** | ADE | **0.70** (0.46-0.83)[b] | - | 0.30 (0.17-0.54) |
| **FSH** | ADE | **0.43** (0.06-0.68) | - | 0.57 (0.32-0.94) |
| **E2** | ACE | 0.02 (0.00-0.50) | **0.65** (0.14-0.79) | 0.32 (0.20-0.52) |
| **T** | ADE | **0.70** (0.43-0.84) | - | 0.30 (0.16-0.5) |
| **Tanner-B** | ACE | **0.72** (0.15-1.00) | 0.23 (0.00-1.00) | 0.05 (0.00-1.00) |
| **Tanner-PH** | ACE | 0.29 (0.00-0.97) | **0.67** (0.07-0.97) | 0.04 (0.00-0.38) |
| Age 12 | | | | |
| **LH** | ADE | **0.65** (0.37-0.81) | - | 0.35 (0.19-0.63) |
| **FSH** | ADE | **0.56** (0.25-0.76) | - | 0.44 (0.24-0.75) |
| **E2** | ACE | **0.54** (0.04-0.89) | 0.28 (0.00-0.71) | 0.19 (0.10-0.37) |
| **T** | ADE | **0.51** (0.05-0.76) | - | 0.49 (0.24-0.95) |
| **Tanner-B** | ACE | 0.21 (0.00-0.33) | **0.69** (0.08-1.00) | 0.10 (0.00-0.20) |
| **Tanner-PH** | ACE | **0.80** (0.22-0.95) | 0.06 (0.00-0.57) | 0.14 (0.04-0.39) |

Abbreviations: CI, confidence interval; E2, estradiol; T, testosterone; , not modeled. Standardized estimates of genetic and environmental influences on hormone levels and Tanner stages at age 9 and 12 years in boys and girls. Genetic influences are reported as broad heritability ($h^2 + d^2$) when variables were modeled in an ADE model and as heritability ($h^2$) when variables were modeled in an ACE model; see column 2. Estimates are derived from a bivariate genetic model. Unstandardized estimates can be found in Supplemental Table 3.2. Bold-typed estimates of genetic and common environmental effects are significantly different from zero.

[a] Based on twin correlations (Supplemental Table 3.1), an ACE model was fitted for estradiol in boys and girls and all Tanner stages in both boys and girls. For the other variables, an ADE model was fitted. Column 3 contains an estimate of additive genetic influences (heritability) in the case of an ACE model and an estimate of additive + nonadditive genetic effects in the case of an ADE model.

[b] Estimate was not significantly different from zero in the data set in which values below the detection limit were excluded.

***Associations between hormone levels***

Correlations between hormone levels differed between age 9 and 12 years and between boys and girls (Figure 3.2). All significant correlations were positive (Table 3.3). In 9-year-old boys, LH correlated only with FSH (Rph = 0.62), whereas at age 12 years, LH correlated with FSH, estradiol, and testosterone (Rph = 0.32, 0.24, 0.49, respectively). In boys only, FSH at age 9 years correlated with all reproductive hormones at age 12 years (Rph = 0.30, 0.51, 0.25, 0.29 for FSH at age 9 years with LH, FSH, estradiol, and testosterone at age 12 years, respectively). In 9-year-old girls, LH correlated with FSH (Rph = 0.64) and estradiol (Rph = 0.31); at age 12 years, LH levels correlated with FSH, estradiol, and testosterone levels (Rph = 0.56, 0.50, 0.40). In girls, LH at age 9 years correlated with LH and estradiol at age 12 years (Rph = 0.40, 0.40).

Bivariate genetic analyses showed that in both boys and girls phenotypic correlations were driven by genetic factors influencing both hormones (Table 3.3). In addition, in boys a common environmental correlation influenced estradiol levels at age 9 and 12 years (Rc = 1.00). In girls a unique environmental correlation was observed between LH and FSH at age 9 years (Re = 0.59) and between LH and estradiol at age 12 years (Re = 0.68).

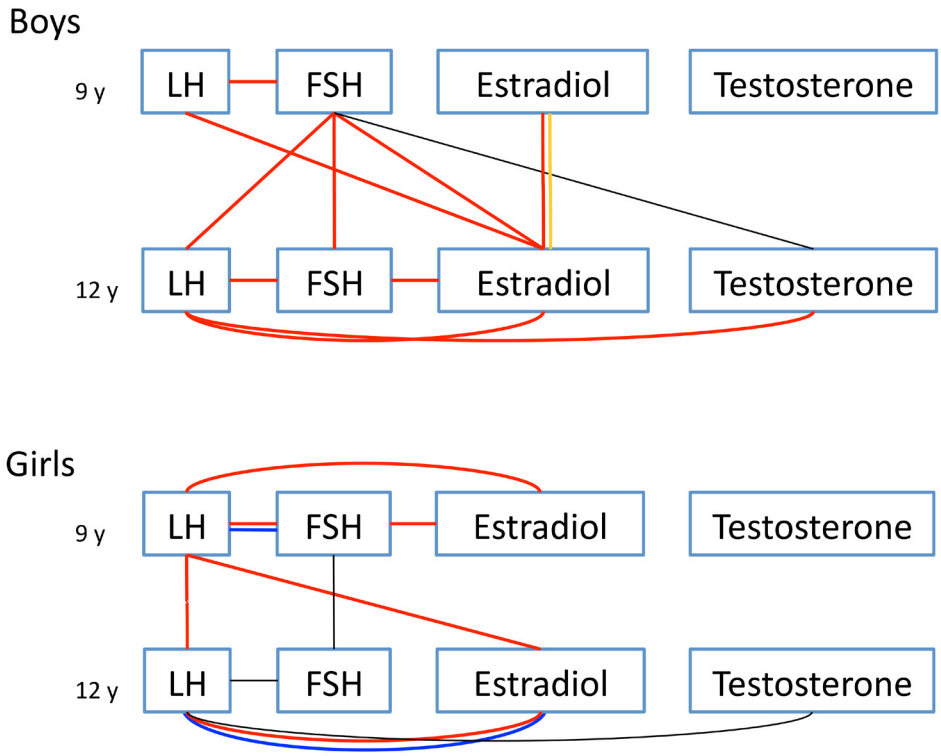


**Figure 3.2** – Significant phenotypic correlations between hormone levels during pubertal development in boys and girls at age 9 and 12 years (see also Table 3). Red: significant genetic correlation (Ra); orange: significant common environmental correlation (Rc); blue: significant unique environmental correlation (Re); black: significant phenotypic correlation that could not be specified to genetic or environmental correlations.

***Associations between hormone levels and Tanner stages***

Significant phenotypic correlations were found between hormone levels at age 9 years and Tanner stages at age 12 years (Table 3.3 and Figure 3.3). In boys, LH at age 9 years correlated with Tanner-P and Tanner-PH development at age 12 years (Rph = 0.29, 0.29); FSH at age 9 years with Tanner-P, Tanner-T, and Tanner-PH development at age 12 years (Rph = 0.34, 0.33, 032); and testosterone at age 9 years with Tanner-PH development at age 12 years (Rph = 0.42). Correlations between hormone levels at age 12 years and Tanner stages at age 12 years were found for LH with Tanner-P (Rph = 0.39) and Tanner-PH development (Rph = 0.33), and for testosterone with Tanner-P, Tanner-T, and Tanner-PH development (Rph = 0.40, 0.44, 0.39) (Figure 3.3).



In girls we found that LH levels at age 9 years correlated with Tanner-B and Tanner-PH development at age 12 years (Rph = 0.56, 0.44) and FSH at age 9 years with Tanner-B development at age 12 (Rph = 0.44). At age 12 years, LH, estradiol, and testosterone levels were correlated with both Tanner-B (Rph = 0.55, 0.70, 0.30) and Tanner-PH development (Rph = 0.46, 0.72, 0.33).

In boys and girls, at both ages, correlations between hormone levels and physical development were mainly driven by genetic factors. The correlation between estradiol at age

**Table 3.3** – Significant associations between hormone levels and Tanner stages in boys and girls during puberty

| | Rph (95%CI) | Model | Rg | Re |
|---|---|---|---|---|
| **Boys** | | | | |
| LH 9 - FSH 9 | **0.62** (0.48-0.73) | ADE-ADE | **0.70** (0.53-0.83) | 0.26 (-0.13-0.60) |
| LH 9 - E2 12 | **0.24** (0.03-0.43) | ADE-ACE | **0.54** (0.33-1.00) | 0.22 (-0.23-0.59) |
| FSH 9 - LH 12 | **0.30** (0.06-0.50) | ADE-ADE | **0.31** (0.05-0.53) | 0.34 (-0.12-0.65) |
| FSH 9 - FSH 12 | **0.51** (0.31-0.66) | ADE-ADE | **0.56** (0.35-0.71) | 0.35 (-0.14-0.70) |
| FSH 9 - E2 12 | **0.25** (0.03-0.45) | ADE-ACE | **1.00** (0.07-1.00) | -0.27 (-0.64-0.25) |
| FSH 9 - T 12 | **0.29** (0.06-0.49) | ADE-ADE | 0.24 (-0.05-0.50) | 0.35 (-0.10-0.67) |
| E2 9 - E2 12[a] | **0.32** (0.12-0.50) | ACE-ACE | **1.00** (0.36-1.00) | -0.40 (-0.68-0.03) |
| LH 12 - FSH 12 | **0.32** (0.10-0.52) | ADE-ADE | **0.32** (0.07-0.53) | 0.03 (-0.39-0.45) |
| LH 12 - E2 12 | **0.24** (0.02-0.44) | ADE-ACE | **0.41** (0.08-1.00) | -0.30 (-0.65-0.17) |
| LH 12 - T 12 | **0.49** (0.28-0.65) | ADE-ADE | **0.55** (0.31-0.74) | 0.09 (-0.35-0.50) |
| FSH 12 - E2 12 | **0.29** (0.08-0.48) | ADE-ACE | **1.00** (0.23-1.00) | -0.06 (-0.49-0.40) |
| LH 9 - Tanner-P 12 | **0.29** (0.03-0.50) | ADE-ACE | **1.00** (0.14-1.00) | -0.15 (-0.82-0.49) |
| LH 9 - Tanner-PH 12 | **0.29** (0.02-0.52)[b] | ADE-ACE | 0.61 (-1.00-1.00) | 0.28 (-0.45-0.82) |
| FSH 9 - Tanner-P 12 | **0.34** (0.09-0.55) | ADE-ACE | **1.00** (0.22-1.00) | -0.11 (-0.61-0.44) |
| FSH 9 - Tanner-T 12 | **0.33** (0.04-0.46) | ADE-ACE | **0.94** (0.59-1.00)[b] | 0.79 (-0.24-0.86) |
| FSH 9 - Tanner-PH 12 | **0.32** (0.06-0.53) | ADE-ACE | **1.00** (0.48-1.00) | 0.01 (-0.62-0.64) |
| T 9 - Tanner-PH 12 | **0.42** (0.18-0.62) | ADE-ACE | **1.00** (0.31-1.00) | -0.24 (-0.68-0.37) |
| LH 12 - Tanner-P 12 | **0.39** (0.10-0.61) | ADE-ACE | **1.00** (0.34 -1.00) | -0.50 (-1.00-0.25) |
| LH 12 - Tanner-PH 12 | **0.33** (0.01-0.59) | ADE-ACE | 0.43 (-1.00-1.00) | -0.04 (-0.71-0.66) |
| T 12 - Tanner-P 12 | **0.40** (0.16-0.60) | ADE-ACE | **1.00** (0.45-1.00) | -0.13 (-0.52-0.32) |
| T 12 - Tanner-T 12 | **0.44** (0.15-0.65) | ADE-ACE | **0.58** (0.20-1.00) | -0.05 (-0.70-0.65) |
| T 12 - Tanner- PH 12 | **0.39** (0.13-0.60) | ADE-ACE | **1.00** (0.23-1.00) | -0.15 (-0.69-0.48) |
| Tanner-P 12 - Tanner-T 12 | **0.70** (0.51-0.83) | ACE-ACE | **1.00** (0.68-1.00) | 0.03 (-0.63-0.59) |
| Tanner-P 12 - Tanner-PH 12 | **0.30** (0.04-0.53) | ACE-ACE | 1.00 (-1.00-1.00) | -0.45 (-0.84-0.14) |

| | | | | |
|---|---|---|---|---|
| **Girls** | | | | |
| LH 9 - FSH 9 | **0.64** (0.51-0.74) | ADE-ADE | **0.71** (0.38-1.00) | **0.59** (0.29-0.79)[b] |
| LH 9 - E2 9 | **0.31** (0.11-0.49)[b] | ADE-ACE | **1.00** (0.28-1.00)[b] | 0.05 (-0.25-0.38) |
| LH 9 - LH 12 | **0.40** (0.19-0.57) | ADE-ADE | **0.50** (0.17-1.00)[b] | 0.15 (-0.24-0.51) |
| LH 9 - E2 12 | **0.40** (0.20-0.57) | ADE-ACE | **1.00** (0.28-1.00) | -0.04 (-0.43-0.36) |
| FSH 9 - E2 9 | **0.23** (0.04-0.41) | ADE-ACE | **1.00** (0.23-1.00) | 0.18 (-0.13-0.47) |
| FSH 9 - FSH 12 | **0.23** (0.03-0.41) | ADE-ADE | 0.48 (-0.03-1.00) | -0.00 (-0.36-0.36) |
| LH 12 - FSH 12 | **0.56** (0.37-0.70) | ADE-ADE | 0.32 (-0.10-0.65)[c] | 0.48 (-0.04-1.00)[c] |
| LH 12 - E2 12 | **0.53** (0.33-0.68) | ADE-ACE | **0.59** (0.17-1.00) | **0.68** (0.38-0.84) |
| LH 12 - T 12 | **0.40** (0.19-0.57) | ADE-ADE | 0.47 (-0.04-1.00) | 0.32 (-0.10-0.65) |
| LH 9 - Tanner-B 12 | **0.56** (0.33-0.73) | ADE-ACE | **1.00** (0.56-1.00) | 0.27 (-0.40-0.78) |
| LH 9 - Tanner-PH 12 | **0.44** (0.19-0.64) | ADE-ACE | **1.00** (0.36-1.00) | -0.42 (-0.81-0.20) |
| FSH 9 - Tanner-B 12 | **0.44** (0.51-0.51) | ADE-ACE | **0.91** (0.13-1.00) | **0.68** (0.17-0.95) |
| LH 12 - Tanner-B 12 | **0.55** (0.29-0.74) | ADE-ACE | **0.78** (0.31-1.00) | 0.28 (-0.71-1.00) |
| LH 12 - Tanner-PH 12 | **0.46** (0.20-0.67) | ADE-ACE | **0.40** (0.22-0.85)[b] | 0.82 (-0.19-1.00)[c] |
| E2 12 - Tanner-B 12[a] | **0.70** (0.52-0.82) | ACE-ACE | 1.00 (-1.00-1.00) | -0.13 (-0.70-0.51) |
| E2 12 - Tanner-PH 12 | **0.72** (0.54-0.91) | ACE-ACE | **1.00** (0.63-1.00) | **0.73** (0.18-0.96) |
| T 12 - Tanner-B 12 | **0.30** (0.07-0.51) | ADE-ACE | **1.00** (0.19-1.00) | -0.28 (-0.83-0.47) |
| T 12 - Tanner-PH 12 | **0.33** (0.09-0.53) | ADE-ACE | 0.35 (-1.00-1.00) | 0.57 (-0.07-0.88) |
| Tanner-B 12 - Tanner-PH 12 | **0.74** (0.59-0.85) | ACE-ACE | 0.77 (-0.60-1.00) | 0.06 (-0.63-0.75) |

Abbreviations: CI, confidence interval; E2, estradiol; T, testosterone. Significant phenotypic correlations (Rph) between hormones at age 9 and 12 years and between hormones at age 9 and 12 years and Tanner stages at age 12 years are given. The extent of overlap in genetic (Rg) and unique environmental influences (Re) acting on both variables are given with their 95% confidence intervals. Bivariate ADE (ADE for both variables), bivariate ACE models (ACE for both variables), and mixed models were fitted, based on the twin correlations per variable. Rg is an estimate of the broad genetic correlation for bivariate ADE models and the additive genetic correlation in other cases. Bold-typed correlations are significant at $P < 0.05$.

[a] In the bivariate ACE models, a common environmental correlation was estimated. It was only significantly explaining the correlation between estradiol at ages 9 and 12 years in boys [Rc = 1.00 (0.30–1.00)] and the correlation between breast development and estradiol at age 12 years in girls [Rc = 1.00 (0.63–1.00)].

[b] Correlation was not significant in the data set in which values below the detection limit were excluded.

[c] Correlation was significant in the data set in which values below the detection limit were excluded.

12 years and Tanner-B development at age 12 years was mainly driven by common environmental factors (Rc = 1.00).

## DISCUSSION

In this paper we described the results of a longitudinal twin study on the development of LH, FSH, and estradiol levels in morning urine, testosterone levels in morning saliva, and Tanner stages in healthy boys and girls at age 9 (at baseline) and 12 years (at follow-up). Because all participants were the same age, variation in hormone levels represents individual developmental differences only and cannot be explained by age differences between the participants. Moreover, the longitudinal aspect allowed us to study the correlations of hormone levels at age 9 years with physical development at age 12 years. We find that at age 9 years, LH and FSH levels in both boys and girls, and testosterone levels in boys, predict secondary sexual characteristics (as described by Tanner stages: breast and pubic hair development in girls; penis, testes, and pubic hair development in boys) at age 12 years. These correlations are largely due to genetic factors that are involved in both early hormone levels and subsequent physical development.

The genetic overlap between genes that regulate hormone levels at age 9 years and physical development at age 12 years implies that certain genes are involved in both the first processes of puberty (as represented by increases in LH and FSH secretion) and subsequent development of secondary sexual characteristics. These significant (genetic) correlations over time suggest that early hormone levels may have a predictive value at the individual level of physical development later during development. This was, however, not the aim of this study because we report correlations that illustrate predictive values on a group level only. Correlations between hormone levels and physical development, both measured at age 12 years, were as expected (Styne, 2007) and correspond with previous reported correlations (Shirtcliff et al., 2009).



To the best of our knowledge, we are the first to measure heritability of reproductive hormone levels in children at age 9 years and again at age 12 years. The only other study to date reported moderate (LH, FSH, estradiol) to high (testosterone) heritability estimates in a group of 35 boys aged between 5 and 11 years (Wang et al., 2004). We find moderate to high heritability estimates for hormone levels at both ages and in both sexes. Interestingly, based on our longitudinal setup, in girls we find a shift from significant common environmental influences at age 9 years to significant genetic influences at age 12 years on estradiol and Tanner-PH development and vice versa for Tanner-B development. Importantly, in boys, heritability estimates and genetic variance (Supplemental Table 3.2) of hormone levels (except for estradiol) increased over time. At both ages, estimates of genetic variance for LH and FSH levels in boys were higher than in girls.

These changes in heritability estimates over time may be related to different stages of development at ages 9 and 12 years: boys develop from prepubertal to early pubertal stages and girls from early to middle pubertal stages. Therefore, processes involved in the maintenance and development of hormone levels and physical posture may differ at age 9 and 12 years, and between boys and girls of the same age [because boys mature at a later age (Marshall and Tanner, 1969; Marshall and Tanner, 1970; Mul et al., 2001)]. HPG axis-related hormones involved in puberty increase and reach peak levels at different developmental stages and are responsible for specific physical changes that come with puberty. For example, LH and FSH levels increase during sleep prior to physical changes but reach a plateau before full maturation (Tanner stage V) (see Supplemental Figures. 3.2 and 3.3) and may even decrease before full maturation is reached (Albertsson-Wikland et al., 1997; Manasco et al., 1997; Sizonenko et al., 1970; Sizonenko and Paunier, 1975; Wennink et al., 1990; Widholm et al., 1974). Testosterone in boys and estrogen levels in both boys and girls start to increase after initiation of physical development (Albertsson-Wikland et al., 1997; Crofton et al., 2002; Gupta, 1975; Raynaud et al., 1993; Shirtcliff et al., 2009; Sizonenko and Paunier, 1975; Wennink et al., 1990; Wennink et al., 1991) and may decrease before full maturation is reached [estrogen in girls (Shirtcliff et al., 2009; Wennink et al., 1991)]. Thus, the increase in reproductive hormone levels during puberty is not only reaching stable adult levels: hormone levels may decrease at the end of puberty, and physical development continues after hormone levels have reached their peak levels. Accordingly, pubertal stages may reflect specific developmental steps toward adulthood. For example, LH levels in Tanner stage II have a more initiation-related function, whereas in Tanner stage IV, LH has a preservative function. This suggests that different stages of puberty are differently influenced by genetic and/or

environmental factors. In addition, earlier it has been suggested that testosterone levels are influenced by age-dependent factors as based on the finding that testosterone levels of fathers and sons did not correlate (Harris et al., 1998). We find that this may also be true for other reproductive hormones. In addition, it has been suggested that FSH is more related to general development, whereas LH is related to pubertal development (Peper et al., 2010). This is in agreement with our current finding that in boys at age 9 years FSH correlated with all other hormones and Tanner stages at age 12 years, whereas in 9-year-old girls LH was correlated with hormone levels and Tanner stages at age 12 years (see Figures. 3.2 and 3.3). Different developmental windows between the sexes may explain the central role of FSH in boys vs LH in girls.



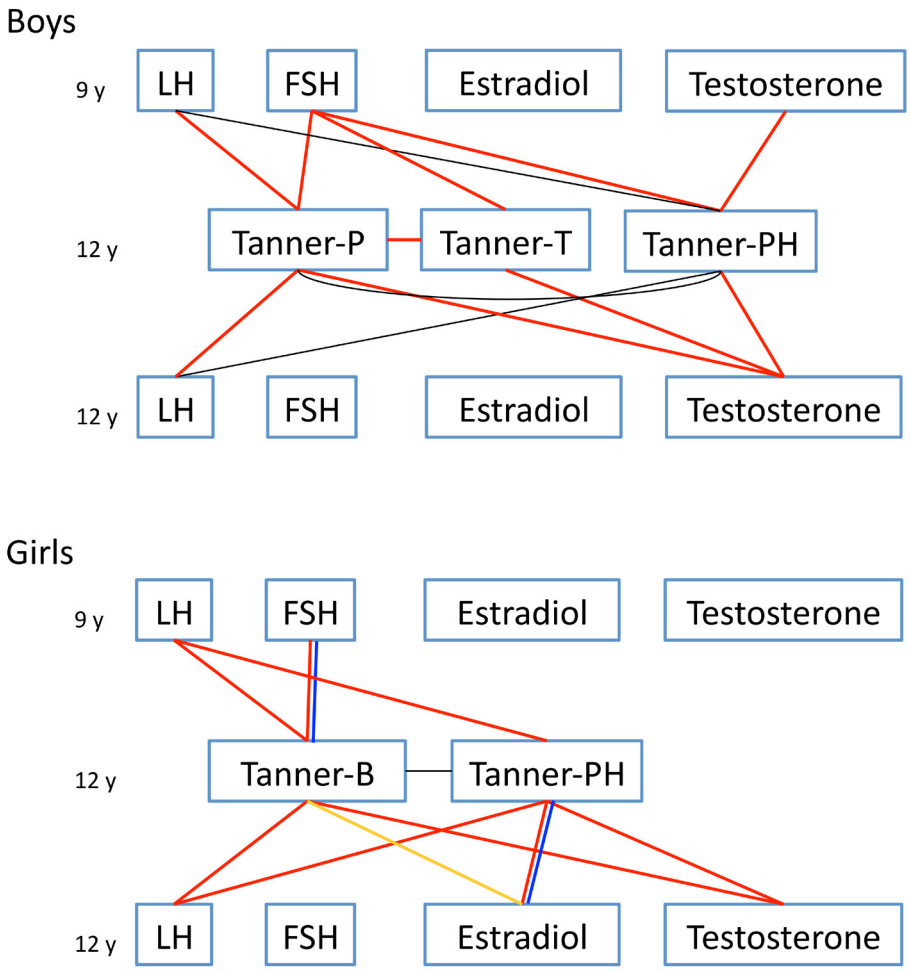


**Figure 3.3** – Significant phenotypic correlations between secondary sexual characteristics at age 12 years (middle) and hormone levels at age 9 years (top) and 12 years (bottom) in boys and girls (see also Table 3.3). Red: significant genetic correlation (Ra); orange: significant common environmental correlation (Rc); blue: significant unique environmental correlation (Re); black: significant phenotypic correlation that could not be specified to genetic or environmental correlations. For the sake of clarity, correlations between hormones are omitted from the figure; see Figure 3.2 for these correlations.

Moreover, in addition to different developmental windows, differences in heritability estimates between boys and girls may be caused by the following 2 explanations. First, the relative influences of genes and environment on pubertal development probably differ between boys and girls because of differences in biological mechanisms. During puberty testosterone (mainly produced by the testes in boys) plays an important role in masculine development, whereas estrogen (mainly produced by the developing follicles in girls) plays an important role in feminine development. However, both hormones are present in both sexes due to conversion of androgens to testosterone in girls and estrogens in boys and because other organs also produce sex hormones (e.g., Burger, 2002; Cameron, 2004; Nelson and Bulun, 2001). In addition to differences in the production of sex hormones, LH and FSH are also differently regulated in boys and girls. Release of both LH and FSH is in part regulated by inhibin-A (in girls) and inhibin-B (in boys and girls). Inhibins A and B are present in different amounts in boys and girls (Foster et al., 2004). This suggests that there are different pathways not only for testosterone and estrogen synthesis but also for LH and FSH secretion. Second, a sex-specific interaction between a hormone and the environment could be involved. Moreover, one can also think of a time-specific hormone-environment interaction. For example, we found that common environmental factors have a high (age 9 years) to moderate (age 12 years, not significant) influence on estradiol levels in girls but are estimated to be low for estradiol levels in boys. Because estradiol plays a different role in girls vs boys, dietary intake (Sowers et al., 2006) or other common environmental factors may have a higher influence on estradiol levels in girls.

The results described in this paper are based on a representative sample of the Dutch population. As such, we did not exclude children being overweight or obese, although excess adiposity may influence hormonal parameters and pubertal onset; extensive literature exists on the (possibly nonlinear) relationship between body mass index (BMI) and menarche (e.g., Biro et al., 2006; Demerath et al., 2004; Mul et al., 2001; Tinggaard et al., 2012). Because in the current cohort, the percentage of children being overweight is representative of the Dutch population, the (genetic) results should be interpreted for the population as a whole, including overweight children.

The results of this study should be interpreted with some caution. The relatively small sample size may potentially lead to instable heritability and environmental estimates. Another source of instability may be differences in developmental stages between children of the same age because small changes in pubertal development may lead to large effects on variance components (Loesch et al., 1995).

In summary, hormone levels at age 9 years are predictive of physical development at age 12 years, in part due to a shared genetic origin. Especially FSH in 9-year-old boys and LH in 9-year-old girls seem to predict hormone levels and secondary sexual characteristics at age 12 years. Variance in hormone levels and physical development was explained by different mechanisms (i.e., genetic or environmental) at ages 9 and 12 years in girls, which stresses the importance of a specific developmental window when studying the heritability of pubertal markers. The third measurement of this longitudinal study is on its way and may provide more information on the development of hormone levels and genetic and environmental factors that influence these processes.

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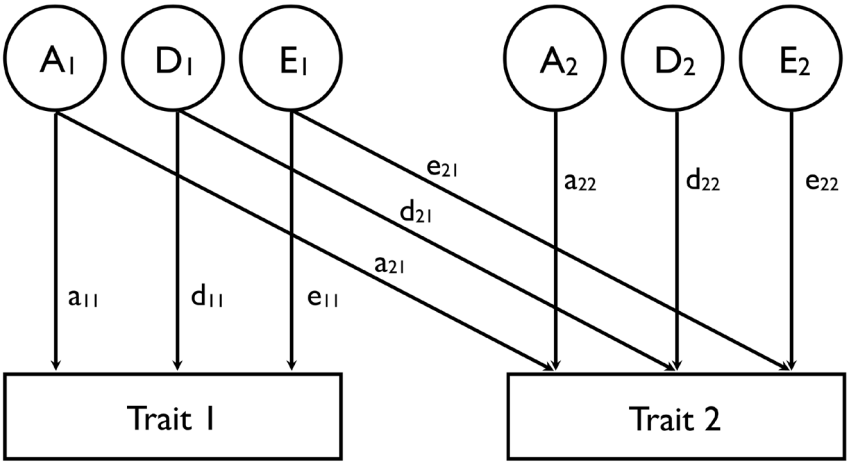
**SUPPLEMENTAL MATERIAL**




**Supplemental Figure 3.1** – The bivariate ADE model. Drawn here is the diagram for only one of the twins. For i in {1,2}, Ai represents the additive genetic factor acting on trait i. Di represents the dominant genetic factor acting on trait i. Likewise, Ei represents the unique environmental factor acting on trait i. The additive and dominant genetic effects were combined to compute broad heritability of the individual traits as $((a_{11})^2 + (d11)^2) / ((a_{11})^2 + (d_{11})^2 + (e_{11})^2)$ for trait 1 and $((a_{21})^2 + (d_{21})^2 + (a_{22})^2 + (d_{22})^2) / ((a_{21})^2 + (d_{21})^2 + (e_{21})^2 + (a_{22})^2 + (d_{22})^2 + (e_{22})^2)$ for trait 2. Further, a (broad sense) genetic correlation between the broad sense genetic factors was computed as $(a_{11} * a_{21} + d_{11} * d_{21})/(\sqrt{((a_{11})^2 + (d_{11})^2)} * \sqrt{((a_{21})^2 + (d_{21})^2 + (a_{22})^2 + (d_{22})^2)})$. The diagram for the other twin is exactly the same but omitted here due to limitations of space. The two diagrams for the twins are connected through the assumptions that follow from the twin model: for monozygotic twins, the factors $A_1$ for twin 1 and twin 2 are fully correlated (r=1.0); and likewise for $A_2$, $D_1$ and $D_2$. For dizygotic twins, the correlations between factors $A_1$ (and $A_2$) for twin1 and twin 2 are by definition equal to 0.5, whereas the correlations between factors $D_1$ (and $D_2$) for twin 1 and twin 2 are by definition equal to 0.25.

**Supplemental Table 3.1** – Twin correlations for hormone levels (a), Tanner stages in boys (b), and Tanner stages in girls (c) for all monozygotic (MZ) and dizygotic (DZ) pairs. Data are presented as correlation (number of complete pairs).

a. Hormone levels

| | LH 9 | FSH 9 | E2 9 | T 9 | LH 12 | FSH 12 | E2 12 | T 12 |
|---|---|---|---|---|---|---|---|---|
| MZ girls | 0.73 (25) | 0.45 (25) | 0.65 (25) | 0.67 (24) | 0.72 (20) | 0.60 (20) | 0.84 (20) | 0.46 (20) |
| DZ girls | 0.02 (21) | 0.17 (21) | 0.72 (21) | 0.40 (19) | 0.46 (14) | 0.31 (16) | 0.57 (16) | 0.08 (16) |
| MZ boys | 0.76 (23) | 0.87 (23) | 0.87 (22) | 0.59 (23) | 0.91 (20) | 0.95 (20) | 0.56 (20) | 0.79 (19) |
| DZ boys | 0.37 (22) | 0.13 (22) | 0.39 (22) | 0.14 (19) | 0.53 (17) | -0.07 (17) | 0.35 (17) | 0.62 (15) |
| DOS boy-girl | 0.51 (19) | 0.42 (19) | 0.26 (20) | 0.22 (20) | 0.21 (15) | 0.28 (15) | 0.16 (15) | 0.32 (15) |

b. Tanner stages in boys *

| | Tanner-P 12 | Tanner-T 12 | Tanner-PH 12 |
|---|---|---|---|
| MZ boys | 0.45 (18) | 0.89 (15) | 0.87 (18) |
| DZ boys | 0.49 (15) | 0.48 (12) | 0.74 (16) |

c. Tanner stages in girls *

| | Tanner-B 12 | Tanner-PH 12 |
|---|---|---|
| MZ girls | 0.94 (19) | 0.90 (17) |
| DZ girls | 0.72 (17) | 0.23 (17) |

MZ= monozygotic; DZ= dizygotic; DOS= dizygotic opposite sex; E2= estradiol; T= testosterone; Tanner-P= penis development; Tanner-T=testes development; Tanner-PH= pubic hair development; Tanner-B= breast development.
* Because of lack of variation in Tanner stages in 9-year-old boy and girls, correlations were computed for age 12 only

**Supplemental Table 3.2** – Unstandardized variance components for genetic, common environmental and unique environmental influences on hormone levels at age 9 and 12 in boys (a) and girls (b).

a. Boys

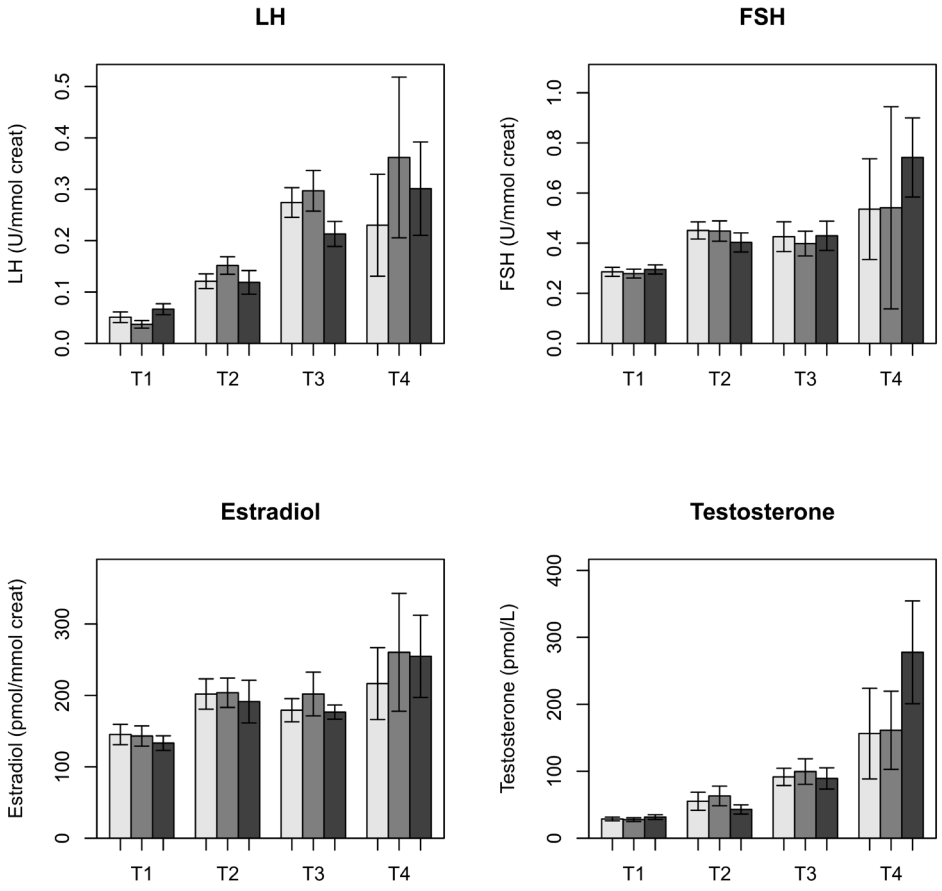
| | | Model[a] | Genetic Variance[a] | Common Environmental Variance | Environmental Variance |
|---|---|---|---|---|---|
| Age 9 | LH | ADE | 0.080 | - | 0.017 |
| | FSH | ADE | 0.059 | - | 0.007 |
| | E2 | ACE | 0.071 | 0.013 | 0.014 |
| | T | ADE | 0.071 | - | 0.040 |
| Age 12 | LH | ADE | 0.182 | - | 0.014 |
| | FSH | ADE | 0.067 | - | 0.004 |
| | E2 | ACE | 0.027 | 0.006 | 0.022 |
| | T | ADE | 0.107 | - | 0.030 |

b. Girls

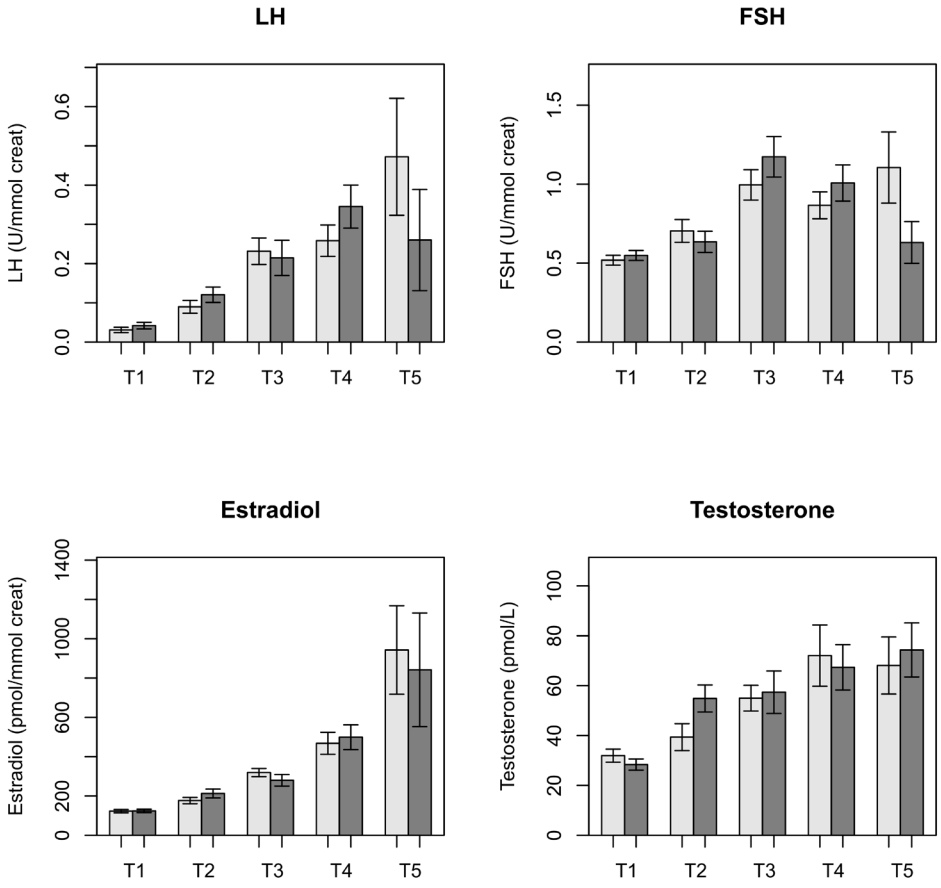
| | | Model[a] | Genetic Variance[a] | Common Environmental Variance | Environmental Variance |
|---|---|---|---|---|---|
| Age 9 | LH | ADE | 0.075 | - | 0.032 |
| | FSH | ADE | 0.031 | - | 0.041 |
| | E2 | ACE | 0.001 | 0.032 | 0.018 |
| | T | ADE | 0.086 | - | 0.033 |
| Age 12 | LH | ADE | 0.143 | - | 0.076 |
| | FSH | ADE | 0.029 | - | 0.022 |
| | E2 | ACE | 0.032 | 0.014 | 0.011 |
| | T | ADE | 0.036 | - | 0.032 |

E2= estradiol; T= testosterone.

[a] Based on twin correlations, an ACE model was fitted for estradiol in boys and girls, and all Tanner scales in both boys and girls. For the other variables, an ADE model was fitted. Column 4 contains an estimate of additive genetic variance in the case of an ACE model, and an estimate of additive + non-additive genetic variance in the case of an ADE model.




**Supplemental Figure 3.2** – Normative hormone levels of 9 and 12-year-old boys categorized according to Tanner stages (T1 – T4). Light grey: penis development; medium grey: testes development; dark grey: pubic hair development. Error bars represent standard error.




**Supplemental Figure 3.3** – Normative hormone levels of 9 and 12-year-old girls categorized according to Tanner stages (T1 – T5). Light grey: breast development; medium grey: pubic hair development. Error bars represent standard error.

Chapter 4

# Longitudinal development of hormone levels and grey matter density in 9 and 12-year-old twins

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**ABSTRACT**

Puberty is characterized by major changes in hormone levels and structural changes in the brain. To what extent these changes are associated and to what extent genes or environmental influences drive such an association is not clear. We acquired circulating levels of luteinizing hormone, follicle stimulating hormone (FSH), estradiol and testosterone and magnetic resonance images of the brain from 190 twins at age 9 [9.2 (0.11) years; 99 females/91 males]. This protocol was repeated at age 12 [12.1 (0.26) years] in 125 of these children (59 females/66 males). Using voxel-based morphometry, we tested whether circulating hormone levels are associated with grey matter density in boys and girls in a longitudinal, genetically informative design. In girls, changes in FSH level between the age of 9 and 12 positively associated with changes in grey matter density in areas covering the left hippocampus, left (pre)frontal areas, right cerebellum, and left anterior cingulate and precuneus. This association was mainly driven by environmental factors unique to the individual (i.e. the non-shared environment). In 12-year old girls, a higher level of circulating estradiol levels was associated with lower grey matter density in frontal and parietal areas. This association was driven by environmental factors shared among the members of a twin pair. These findings show a pattern of physical and brain development going hand in hand.



**INTRODUCTION**

Pubertal sex steroids have organizational and activating effects on brain structure, and on structural and functional connectivity (see reviews by Ladouceur et al., 2012; Peper et al., 2011a; Peper et al., 2011b; Sisk and Zehr, 2005). The study of healthy pubertal brain development is considered to be important in the light of the age of onset of psychiatric diseases. Several psychiatric diseases show their first symptoms during puberty and adolescence (Kessler et al., 2007) and it is thought that in this sensitive period, aberrant brain development occurs (Insel, 2010). Gonodal hormones, as well as adrenal hormones, are thought to be important for teenage mental health (Marceau et al., 2015).

Several studies have found associations between pubertal hormones and brain structure (Bramen et al., 2011; Bramen et al., 2012; Herting et al., 2012; Herting et al., 2014; Koolschijn et al., 2014; Neufang et al., 2009; Nguyen et al., 2013a; Nguyen et al., 2013b; Peper et al., 2008; Peper et al., 2009a; Perrin et al., 2008). However, findings differ considerably between studies. Cross-sectionally, testosterone levels have been positively associated with whole brain size (boys; Peper et al., 2009a) and white matter volume (boys; Perrin et al., 2008; both sexes; Herting et al., 2014), amygdala (sexes combined; Neufang et al., 2009; sex × testosterone × time interaction; Herting et al., 2014) and hippocampal volume (sexes combined; Neufang et al., 2009) and have been negatively associated with cortical grey matter (girls; Bramen et al., 2011) and cortical volume (sexes combined; ACC and OFC Koolschijn et al., 2014). Changes in testosterone levels have been associated with both thinning of the cortex (boys) and thickening of the cortex (girls) (Nguyen et al., 2013a). The few studies measuring estradiol and brain structure have found higher estradiol levels associated with smaller grey matter density and grey matter volume (girls; Peper et al., 2009a), smaller ACC volume (both boys and girls; Koolschijn et al., 2014), larger parahippocampal volume (sexes combined; Neufang et al., 2009) and smaller white matter volume and right amygdala (girls, Herting et al., 2014). Zooming in on white matter, a positive association between fractional

anisotropy and testosterone has been found in boys and a negative associating between estradiol and fractional anisotropy in girls (Herting et al., 2012). These findings are not easily summarized, possibly due to the fast changes that adolescents undergo in both hormone levels and brain structure during adolescence. The strong interaction between age and pubertal development and differences in timing of puberty between the sexes may also play a role. Longitudinal studies combining pubertal development and brain maturation may provide a clearer picture. Thus far, these studies have mainly focused on testosterone (Herting et al., 2014; Nguyen et al., 2013a); in combination with dehydroepiandrosteron (Nguyen et al., 2013b), and testosterone receptors (Raznahan et al., 2010). The effect of estradiol on brain structure has been studied in a longitudinal way only recently (Herting et al., 2014): estradiol predicted white matter and right amygdala growth, and grey matter volume decrease in both boys and girls.

Here we investigate the associations of puberty-related hormones and brain structure in a longitudinal twin sample at ages 9 and 12 years. We acquired MRI brain images and assessed circulating luteinizing hormone (LH), follicle stimulating hormone (FSH), and estradiol levels in urine, and testosterone levels in saliva. Our previous work in this sample has shown that genetic factors influence (a) gray and white matter density of the brain at age 9 (Peper et al., 2009b); (b) fractional anisotropy in the main fiber tracts at age 9 and 12 (Brouwer et al., 2010a; Brouwer et al., 2012); (c) the extent to which brain volume (van Soelen et al., 2013) and cortical thickness (van Soelen et al., 2012a) changes between the age of 9 and 12 years. Variation in hormone levels were also mainly driven by genes at both ages, the exception being estradiol in girls, which seems to be influenced by a common environmental factor (Koenis et al., 2013). At age 9, a positive association between luteinizing hormone (LH) and white matter density in areas covering the splenium, left cingulum and bilateral middle temporal gyrus was seen in both sexes combined, but no correlations between the other hormones and the brain were found (Peper et al., 2008). Three years later at age 12, variation in hormone levels increased substantially, and more children had entered puberty. Here we explore the relationship between pubertal hormones and grey matter density in a genetically informative longitudinal design. We expect that higher hormone levels are associated with a more mature brain, i.e. lower cortical grey matter density. Decreasing cortical gray matter density is a process that occurs throughout childhood and adolescence. Cortical regions associated with basic functions such as vision and motor skills mature earlier than regions associated with higher order functions (Gogtay et al., 2004). However, the biological mechanisms underlying cortical thinning as observed with MRI are not clear: Cortical thinning is thought to reflect either myelination of fiber bundles close to the cortex (Paus et al., 2001), or synaptic pruning. The latter idea is based on the use-it-or-lose-it principle: dendrites and synapses that are not essential may be removed during development (Huttenlocher et al., 1982).

The twin sample allows (Boomsma et al., 2002) assessing whether associations among measures of grey matter density in the brain and hormone levels can be attributed to variations in the genome (genetic pleiotropy) or can be attributed to environmental factors that influence both phenotypes.

## METHODS

### *Subjects*

Twins families from the Netherlands Twin Registry (van Beijsterveldt et al., 2013) were recruited for the BrainSCALE project (van Soelen et al. 2012b). At the first assessment at age 9 years (mean 9.2, SD 0.11 years), 190 twin subjects (99 females/91 males; 21 complete MZF pairs, 16 complete DZF pairs, 17 complete MZM pairs, 16 complete DZM pairs; 19 females and 14 males part of an opposite sex twin pair) underwent an extensive MRI protocol at the University Medical Center Utrecht, as was described before (Peper et al., 2009a; Peper et al., 2009b; van Soelen et al., 2012b). Exclusion criteria consisted of having a pacemaker, any metal material in the head and a known history of any psychiatric illness or major medical condition. Urine and saliva samples were collected on two consecutive weekdays for assessment of hormonal levels. Three years later, 125 participants returned for follow-up measurements at the University Medical Center Utrecht (mean age of twins 12.1, SD 0.24 years) (59 females/66 males; 10 complete MZF pairs, 10 complete DZF pairs, 13 complete MZM pairs, 8 complete DZM pairs, 11 females and 13 males part of an opposite sex twin pair). 113 children were scanned twice (60 boys, 53 girls). Zygosity of same-sex twins was confirmed by genome-wide SNP data. Both parents and children 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 (CCMO) and was in agreement with the Declaration of Helsinki (Edinburgh amendments).



### *Hormonal measurements*

At both assessments, luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol levels were determined in first morning urine on two consecutive days, by means of competitive immunometric luminescence assays (Architect, Abbott Laboratories, Diagnostic Division Abbott Park, Illinois, USA). Testosterone levels were obtained from saliva sampled after waking up, which were collected on the same days as the morning urine samples (competitive immunometric luminescence assay, IBL Hamburg, Germany). The lower limits for detection were 0.11 U/l (units/liter) for FSH, 0.1 U/l for LH, 150 pmol/l for estradiol and 11 pmol/l for testosterone. Hormonal levels were assessed by the endocrinological laboratory of clinical chemistry of the VU Medical Center in Amsterdam. At age 9, none of the subjects had attained menarche. At age 12, 16 girls had attained menarche of which 1 reported a regular cycle. None of the participants used oral contraceptives.

LH, FSH and total estradiol levels were divided by creatinine levels, thus correcting for variations in urine excretion rate (Kesner et al., 1998). All hormonal levels were averaged over the 2 days (if present for both days). Some children had hormone levels below detection limit on one or both days (Table 4.1). When data were available on 1 day only, these were entered into the analyses. The distributions of these levels were highly skewed to the left and log-transformed data were entered in all subsequent analyses. After transformation, hormonal data satisfied Mardia's test for multivariate normal distributions (p-skewness 0.22/0.19, p-kurtosis 0.29/0.06 in girls and boys, respectively).

Tanner stages were assessed using the Tanner scales of development (Tanner, 1962) through a physical exam by a trained researcher. If children were uncomfortable with the

procedure, they were asked to point out their status on photographs, which were accompanied by an oral explanation of the researcher (only at age 12, 30% boys, 15% girls). A subset of children completed a self-report and a physical exam, and ICC values ranged between 0.73 and 0.77 except for genital development in boys (0.44). When available, we used the data as assessed by the researcher. The genetic analyses of variation in the hormone levels and associations between hormones levels and Tanner stages have been published elsewhere (Koenis et al., 2013).

**Table 4.1 –** Demographics

| | **First assessment Age 9** | | **Second assessment Age 12** | |
|---|---|---|---|---|
| | **Female** | **Male** | **Female** | **Male** |
| Number of twin subjects | 99 | 91 | 59 | 66 |
| Mean age [years, (range)] | 9.2 (9.0–9.6) | 9.2 (9.0–9.6) | 12.1 (11.7–13.1) | 12.1 (11.7–13.1) |
| MZ subjects (complete pairs) | 43 (21) | 39 (17) | 26 (10) | 30 (13) |
| DZ subjects (complete pairs)[a] | 56 (16) | 52 (16) | 33 (10) | 36 (8) |
| Total brain volume (ml) (SD) | 1264.1 (85.5) | 1407.5 (92.8) | 1272.1 (96.0) | 1423.5 (91.7) |
| Cerebral grey matter volume (ml) (SD) | 673.1 (49.4) | 737.1 (50.4) | 655.4 (54.8) | 725.7 (50.3) |
| Cerebral white matter volume (ml) (SD) | 430.7 (39.7) | 493.8 (45.0) | 454.4 (45.4) | 520.8 (46.7) |
| LH (U/mmol creatinine) (SD)[b] | 0.02 (0.01) | 0.02 (0.03) | 0.21 (0.22) | 0.18 (0.14) |
| Number of subjects below detection limit on 0/1/2 days | 28/20/51 | 33/26/33 | 53/3/3 | 62/3/1 |
| FSH (U/mmol creatinine) (SD)[b] | 0.47 (0.33) | 0.27 (0.18) | 0.92 (0.51) | 0.43 (0.33) |
| Number of subjects below detection limit on 0/1/2 days | 98/0/0 | 90/1/0 | 59/0/0 | 66/0/0 |
| Estradiol (pmol/mmol creatinine) (SD)[b] | 120.8 (81.3) | 135.5 (124.9) | 344.2 (238.1) | 203.2 (136.1) |
| Number of subjects below detection limit on 0/1/2 days | 97/2/0 | 87/2/0 | 58/1/0 | 66/0/0 |
| Testosterone (pmol/l) (SD)[b] | 31.1 (22.7) | 26.1 (26.8) | 59.6 (40.1) | 68.2 (82.4) |
| Number of subjects below detection limit on 0/1/2 days | 85/7/3 | 82/6/3 | 58/1/0 | 57/6/3 |

Abbreviations: MZ, monozygote; DZ, dizygote; LH, luteinizing hormone; FSH, follicle stimulating hormone
[a]At age 9, 19 females/14 males were part of an opposite-sex twin pair. At age 12, 11 females/13 males were part of an opposite-sex twin pair
[b] Means and standard deviations are given for the group of children that produced levels above the detection limits. See Koenis et al., 2013 for more information on the hormone levels and their (genetic) associations

### *MRI acquisition and voxel based morphometry*

For both assessments, structural magnetic resonance images were made on a 1.5 Tesla Philips Achieva scanner (Philips, Best, the Netherlands) using the same protocol. A three-dimensional T1-weighted scan (Spoiled Gradient Echo; TE = 4.6 ms; TR = 30 ms; flip angle 30°; 160–180 contiguous coronal slices of 1.2 mm; in-plane resolution 1 × 1 $mm^2$; acquisition matrix 256 × 256) of the whole head was acquired of each subject. Intracranial masks were obtained as described in (van Soelen et al., 2013). Each voxel in the intracranium was segmented into fractions of grey matter, white matter and cerebrospinal fluid using partial volume segmentation (Brouwer et al., 2010b). This segmentation was used to obtain estimates of total brain volume, and grey and white matter volume of the cerebrum. After segmentation, images were blurred with a 3D Gaussian kernel with full-width half-max of 8 mm and

subsequently warped into model space: a linear transformation to the model brain [based on optimizing a joint entropy mutual information metric (Maes et al., 1997)] was followed by nonlinear transformations with a precision up to 4 mm (ANIMAL, Collins et al., 1995). The model brain was created from the T1-weighted images of the twins and their older siblings in this study at baseline, as was described before in (Peper et al., 2008). Finally, voxels were resampled to 2 × 2 × 2.4 $mm^3$ to increase statistical power. The remaining images represent the local presence, or so-called density of grey matter. Magnetic resonance imaging and post-processing of the MRI data was done at the University Medical Center, Utrecht.

***Statistical analysis***

Analyses were carried out for boys and girls separately, as hormone production and subsequent physical (brain) changes may be assumed to be sex-specific. All available data were entered into the analyses, regardless whether subjects participated at age 9, age 12, or both. All analyses were implemented in OpenMx (Boker et al., 2011). Missing data were handled using the full information maximum likelihood procedure. We estimated correlations between changes in hormone level and changes in grey matter density in each voxel in a longitudinal design (Figure 4.1), for each hormone and for boys and girls separately. In this model, the variance of the latent change variable $V_{chGM}$ "change of grey matter density" is modeled as $Var_{(density\ age\ 9)} + Var_{(density\ age\ 12)} - 2Cov_{(density\ age\ 9,density\ age\ 12)} = a_2^2 + c_2^2 + e_2^2 + a_4^2 + c_4^2 + e_4^2 - 2(a_2 * r_{g(2,4)} * a_4 + c_2 * r_{c(2,4)} * c_4 + e_2 * r_{e(2,4)} * e_4)$. Variance of latent change in hormone level $V_{chH}$ was defined likewise. The covariance between change in grey matter density and change in hormone was modeled as $Cov_{(chGM,chH)} = Cov_{(hormone\ age\ 12,\ density\ age\ 12)} + Cov_{(hormone\ age\ 9,\ density\ age\ 9)} - Cov_{(hormone\ age\ 9,\ density\ age\ 12)} - Cov_{(hormone\ age\ 12,\ density\ age\ 9)} = a_3 * r_{g(3,4)} * a_4 + c_3 * r_{c(3,4)} * c_4 + e_3 * r_{e(3,4)} * e_4 + a_1 * r_{g(1,2)} * a_2 + c_1 * r_{c(1,2)} * c_2 + e_1 * r_{e(1,2)} * e_2 - a_1 * r_{g(1,4)} * a_4 - c_1 * r_{c(1,4)} * c_4 - e_1 * r_{e(1,4)} * e_4 - a_2 * r_{g(2,3)} * a_3 - c_2 * r_{c(2,3)} * c_3 - e_2 * r_{e(2,3)} * e_3$. We first tested whether change in hormone level was associated with change in grey matter density by constraining the correlation $Cov_{(chGM,chH)}/sqrt(V_{chGM} * V_{chH})$ to be zero. Minus twice the difference of log-likelihoods of these models was distributed as a Chi-square distribution with one degree of freedom. Then, we tested in the same longitudinal model whether the hormone and density were associated at age 12 by constraining $Cov_{(hormone\ age\ 12,\ density\ age\ 12)}$ to equal zero. Associations at age 9 have been published before (Peper et al., 2008). For each of these analyses, we corrected for multiple comparisons using the False Discovery Rate (FDR) (Genovese et al. 2002) at an FDR level of 0.05.

For the associations that reached whole brain significance (i.e. significant after FDR correction for multiple comparisons), we investigated whether the association was driven by genes or environment. Twin data allows for disentanglement of these factors, which follows from comparing monozygotic (MZ) and dizygotic (DZ) twin pairs. MZ pairs have the same genetic make-up, while DZ pairs on average share 50% of their segregating genes. If cross-trait/cross-twin correlations (e.g. the correlation between grey matter density of twin 1 and hormone level of twin 2) are larger in MZ twins than in DZ twins, there is a genetic component to the association. If this correlation is less than twice as large in MZ twins compared to DZ twins, there is a common environmental correlation between the two traits. Finally, it is possible that a unique environmental component drives the association between two traits. In that case, there is a correlation between the two traits, but only within persons (and not between members of a twin pair. The extent to which the genetic or environmental

correlations explain the phenotypic correlation between two traits, depends on the etiology of the traits, as for example, the genetic correlation is weighted by the square root of the heritabilities. We therefore also computed the rph-a, rph-c and rph-e: rph-a can be seen as the correlation that would have been observed if only genetic factors play a role and is defined as the covariance between two trait due to genetic factors, divided by the sqrt of variances of those two traits. In case of the change between hormone level and change in grey matter density, this equals $(a_3*r_{g(3,4)}*a_4 + a_1*r_{g(1,2)}*a_2 - a_1*r_{g(1,4)}*a_4 - a_2*r_{g(2,3)}*a_3)/sqrt(V_{chGM}*V_{chH})$. rph-c and rph-e are defined likewise (Toulopoulou et al., 2007).

As a post hoc analysis, we investigated whether our results could be driven by the (small) differences in age at each measurement or scanning interval, we repeated the analyses in whole brain significant voxels with a correction for age at each measurement separately.

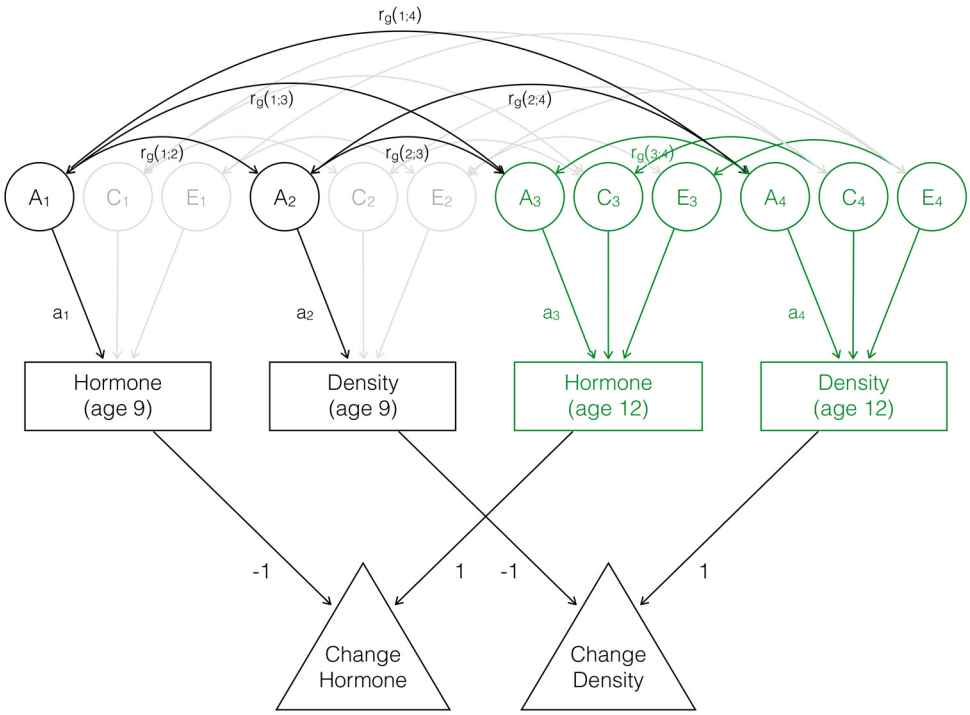


**Figure 4.1** – Path diagram representing the longitudinal genetic model fitted to the data collected at age 9 and 12. For simplicity, only the diagram for twin 1 is shown. The genetic components A1 to A4 of twin 1 are connected to those of twin 2 with a correlation of 1 in monozygotic twins, and 0.5 in dizygotic twins. The common environmental components C1 to C4 of twin 1 are connected to those of twin 2 with a correlation of 1 by definition. For visualization purposes, the common and unique environmental correlations and paths are not labeled. The submodel that was used to investigate the association between estradiol and grey matter density at age 12 only is colored green.

**RESULTS**

Demographic characteristics can be found in Table 4.1. Hormone levels increased for all hormones in both boys and girls ($p < 0.001$; see Figure 4.2). Correlations between hormones have been described previously in (Koenis et al., 2013). In summary, in boys of age 9, LH correlated with FSH ($r = 0.62$). At age 12, LH correlated with all the other hormones ($r \sim 0.5$). In girls of age 9, LH, FSH and estradiol correlated with each other (ranging from 0.2 to 0.6). At age 12, LH correlated with all other hormones ($r \sim 0.45$) (Koenis et al., 2013). The distribution of Tanner stages can be found in Supplemental Figure 4.1. Cross-sectionally, there were no significant correlations between hormone levels and total, grey, or white matter volume at either age 9 or age 12.



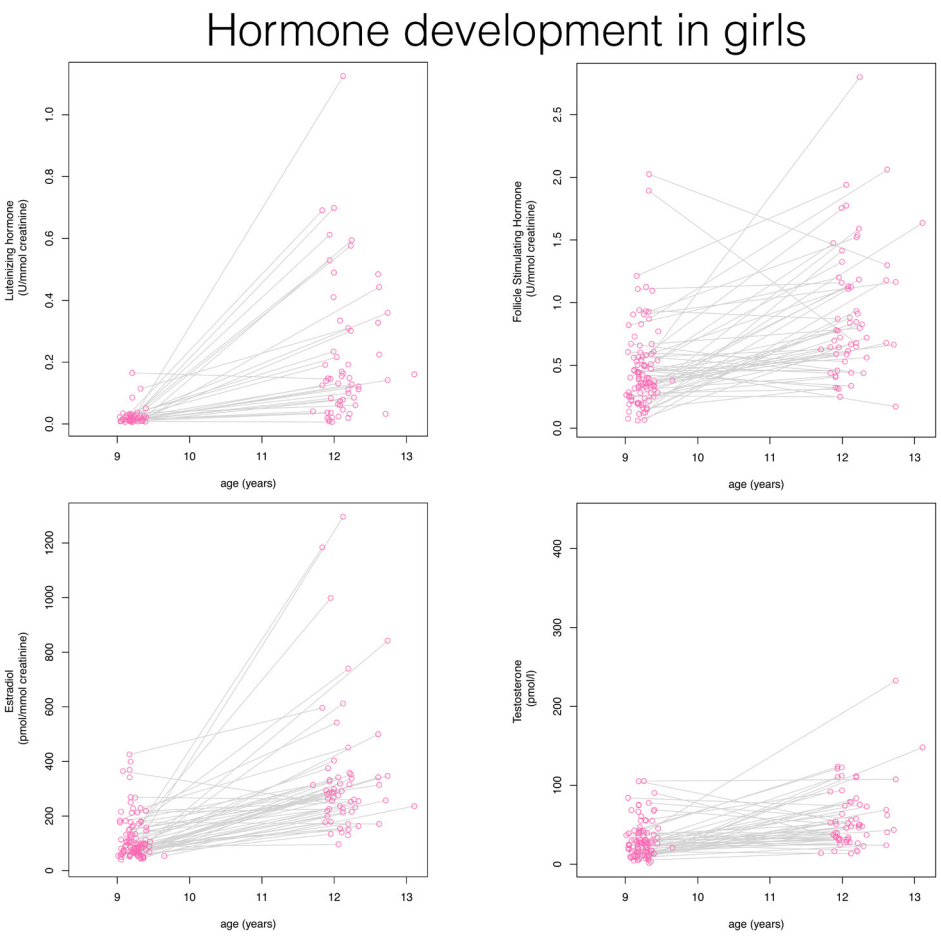


**Figure 4.2a** – Changes in hormone levels over time in girls

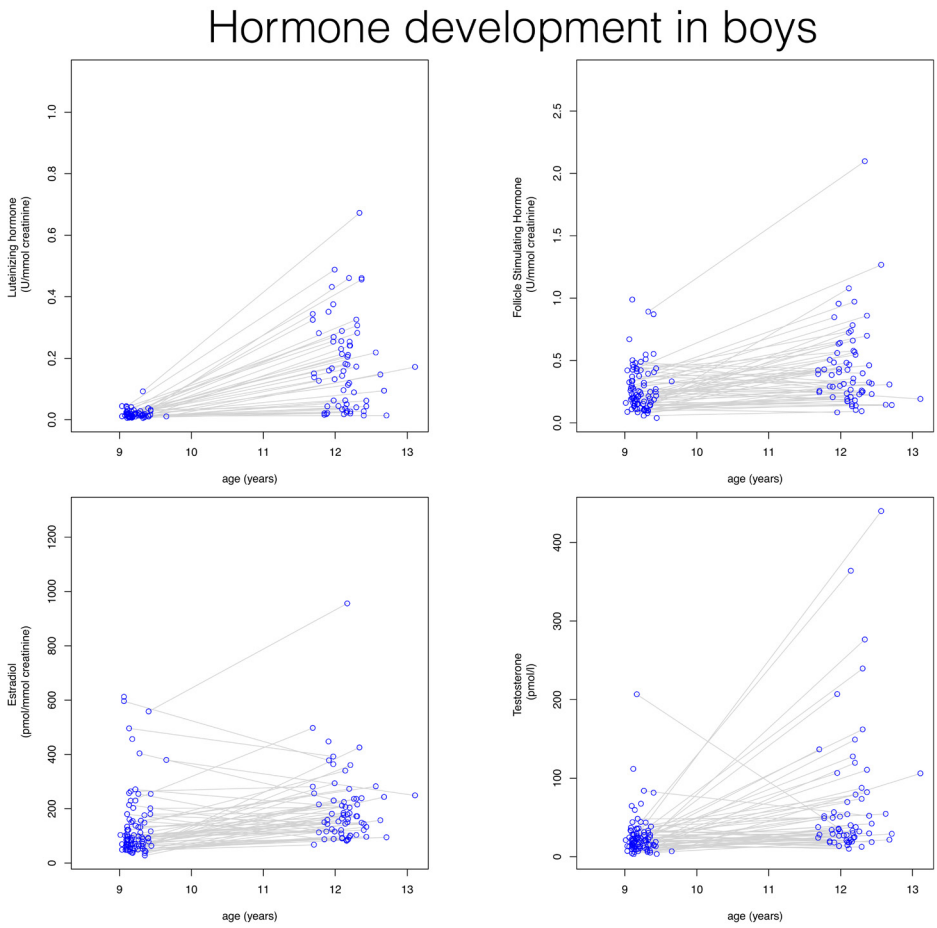


**Figure 4.2b** – Changes in hormone levels over time in boys

***Longitudinal analysis change in hormone levels and change in grey matter density***

On average, hormone levels increased from ages 9 to 12, while grey matter density decreased on average. In girls, there was a significant positive association between change in FSH levels and change in grey matter density in areas covering the left hippocampus, left (pre)frontal areas, right cerebellum, and left anterior cingulate and precuneus (Figure 4.3; average correlation 0.45, Critical $\chi^2$ = 11.60, FDR corrected at alpha level of 0.05, df = 1). Within these voxels, we investigated whether there was a shared genetic, common environmental or unique environmental component explaining both changes in FSH and changes in grey matter density.

In 58% of these voxels, the association was driven by an environmental factor unique to the individual, influencing both grey matter density changes and changes in FSH (Critical $\chi^2 = 4.76$, FDR corrected at alpha level of 0.05, df = 1). In the remaining 42% of the voxels, individual contributions of genetic or environmental sources contributing to the correlation between change in estradiol and change in grey matter density did not reach whole brain significance. When correcting for the small age differences within either measurement, conclusions did not change.

There were no whole-brain significant correlations between the change in grey matter density and the change in hormone levels for the other hormones in girls, nor were any significant associations found in boys.



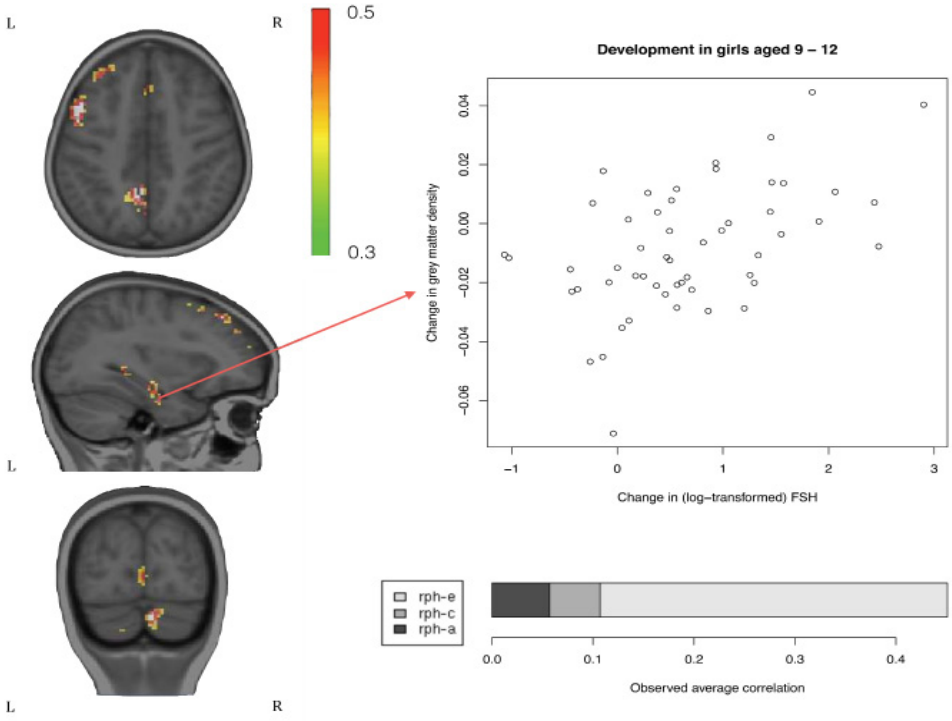


**Figure 4.3** – Significant positive associations between changes in grey matter density and changes in FSH levels in girls (FDR corrected; alpha = 0.05). Clusters of positive associations were found in the left hippocampus (middle panelinset), left frontal areas (top andmiddle panel), left precuneus (top and bottom panel), and right cerebellum (bottom panel). Bottom right the average correlation between changes in grey matter density and changes in FSH split up into the contribution of genetic influences shared by the two phenotypes (rph-a), common environmental influences shared by the two phenotypes (rph-c) and contribution of unique environmental influences shared by the two phenotypes (rph-e). These three add up to the observed phenotypic correlation.

***Associations between hormone levels and grey matter density at age 12***

We found significant negative correlations between grey matter density and estradiol levels in girls at age 12, in mainly left frontal and parietal cortical areas (Figure 4.4; Average correlation -0.47, Critical $\chi^2 = 12.58$, FDR corrected at alpha level of 0.05; df = 1). Within these voxels, we investigated whether there was a shared genetic, common environmental or unique environmental component explaining both estradiol level and grey matter density, but these could not be disentangled, or rather, effects were not so large that they survived the correction for multiple comparisons. The nature of the results did not change when correcting for the small age differences within either measurement.

When we investigated this association in a model with fewer degrees of freedom, using a bivariate model incorporating data at age 12 only (submodel in Figure 4.1), significant associations between estradiol and grey matter density at age 12 were found in the same frontal and parietal areas, but were much more widespread. A significant contribution of common environmental influences could be determined (9% of the voxels, critical $\chi^2 = 8.05$, FDR corrected at alpha level of 0.05; Supplemental Figures 4.2 and 4.3). In the remaining 91% of the voxels, individual contributions of genetic or environmental sources contributing to the correlation between estradiol and grey matter density did not reach whole brain significance.

No other associations between hormone levels and grey matter density were found at age 12 in girls. No associations were found in boys.



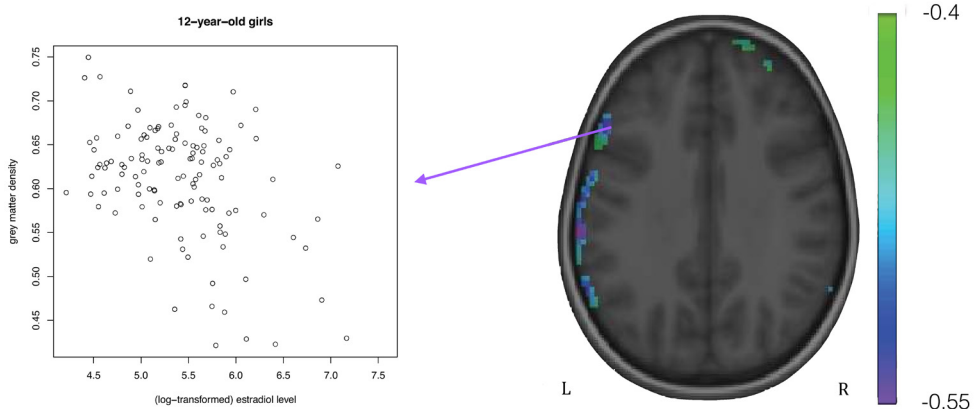


**Figure 4.4** – Significant negative associations between grey matter density and estradiol levels in girls at age 12 (FDR corrected; alpha = 0.05). Associations were predominantly found in (left) frontal and parietal areas.

**DISCUSSION**

In this study we measured the influence of pubertal hormones on brain structure between 9 and 12 years of age and the extent to which these associations might be due to genetic and environmental influences. The main finding is that, in girls between the ages of 9 and 12, changes in FSH were associated with changes in areas covering the left hippocampus, left (pre)frontal areas, right cerebellum, and left anterior cingulate and precuneus. These associations were driven by unique environmental factors. Moreover, in 12-year-old girls, higher estradiol levels were associated with lower grey matter density, mainly in frontal and parietal areas. The associations between estradiol and grey matter densities were driven by common environmental influences.

Apart from our earlier work in which we showed no associations between FSH and the brain at ages 9–15 (Peper et al., 2008; Peper et al., 2009a), there has been little investigation of FSH in the healthy developing brain. Here, we found changes in FSH to be associated with changes in grey matter density, most interestingly in the left hippocampus. Hippocampal volumes have been shown to associate with Tanner stage in a nonlinear fashion in girls (Goddings et al., 2014) but not with sex steroid levels (Herting et al., 2014). Our results now suggest that maybe FSH is responsible for hippocampal growth in girls in the early phases of puberty. At this time, the environmental factors that explain both changes in FSH and grey matter density are unknown. There is a genetic variant for a FSH receptor gene that strongly influences the onset of puberty (Hagen et al., 2014), but our findings suggest genetic variants influencing FSH do not also influence the changes in grey matter density. Girls that are developing fast (indicating by a large increase in FSH) also obtain new (social) behavior. Indeed, early maturing girls are hypothesized to have the most adjustment difficulties because they are physically most deviant from their peers or alternatively, because they have the shortest period of time to adjust to new social and behavioral norms (Negriff and Susman, 2011). Early maturation in girls has been associated with risk for adult psychopathology (Graber, 2013) but also with a lower social competence (Westling et al., 2012). We may speculate that these social demands are reflected in the brain.

At age 12, we find that estradiol is negatively associated with grey matter density in girls. The associations are most prominent in the frontal and parietal brain areas. A similar pattern was seen in the older sisters of the twins in our cohort (Peper et al., 2009a). Both a higher estradiol level and less grey matter density (or cortical thinning) are signs of maturation. Here we show that the estradiol and grey matter density are related already at age 12. These findings cannot be attributed to influences of age, both of which are clearly associated with estradiol and grey matter density, since all the individuals included in the current cohort were 12 years at the time of the assessment. It is an interesting observation that much more voxels reached significance when we considered a model incorporation data from age 12 only. The longitudinal model is more flexible in the sense that there are more parameters, hence the drop in log-likelihood will be probably be smaller, leading to a smaller number of voxels that survive FDR correction for multiple comparisons.

The association between grey matter density and estradiol was mainly explained by common environmental factors shared by twins raised in the same family. As to the environmental factors that may explain the link between estradiol levels and gray matter density in girls we can only speculate. Possible candidates for this environmental source are nutrition,

as body mass index has been shown to advance the start of puberty (Wagner et al., 2012), or father absence, the effect of which is moderated by ethnicity and income (Deardorff et al., 2011). Although the evidence for a shared genetic background for pubertal brain development and pubertal hormones is limited in our cohort, there are certainly genes that influence both processes. Recent studies showed effects of the number of a polymorphic trinucleotide repeat in a gene encoding for the androgen receptor in both white (Perrin et al., 2008) and grey matter (Raznahan et al., 2010). The latter study showed that a lower number of repeats, i.e. the more efficient variant, was associated with a more masculine cortical maturation pattern.

It remains an open question whether FSH or estradiol cause these changes in grey matter density. Although both FSH receptors and estradiol receptors are expressed in the brain (Hawrylycz et al., 2012), these receptors are not overly expressed in the regions in which we now observe associations between grey matter density and these hormones. Indeed, it may be that an earlier pubertal marker triggers the full cascade of both hormonal and brain changes. Examples of these include gonadotropin releasing hormone (GnRH), kiss-peptins that stimulate GnRH (Smith and Clarke, 2007) or other factors triggering the growth spurt such as growth hormone, or insulin-like growth factor (Styne, 2003). Another promising candidate is the steroid hormone dehydroepiandosterone (DHEA) which has been shown to associate with cortical thickness, especially in the age range between 4 and 13 years (Nguyen et al., 2013b). These authors also show an interaction effect of DHEA and testosterone on cortical thickness. It is very likely that a combination of different hormonal influences is leading to the brain changes we observe in puberty.

The idea that another hormone is implicated in both brain changes and physical maturation during puberty may also explain why thus far, no associations between estradiol and decreases of grey matter density have been found in boys. Another explanation of the absence of such an association in boys may be the delayed pubertal maturation of boys compared to girls. This delay occurs both in physical development (Mul et al., 2001) and in brain maturation (Raznahan et al., 2010) as characterized by cortical grey matter changes. The distribution of the Tanner stages (Supplemental Figure 4.1) shows that this is indeed the case in our cohort. The observation that the boys in our cohort are simply "too young" to show much variations in levels of hormones, specifically testosterone, may also explain the differences between our study and those of others that show associations between testosterone and the brain in boys (e.g. Nguyen et al., 2013a; Nguyen et al., 2013b; Raznahan et al., 2010). That said, levels of sex steroids are different in boys and girls once they proceed into puberty, and this may be used as an argument that not the sex steroids, but earlier (pubertal) hormones are responsible for grey matter maturation. Another finding in favor of the view that pubertal brain changes are not driven by sex steroid production is the finding that at age 9, the children in our cohort with the first signs of secondary sexual characteristics showed decreased grey matter density in (pre)frontal and parietal areas (Peper et al., 2009b), while direct associations between the sex steroids and brain structure were not present at that time (Peper et al., 2008).

There are several limitations to take into account when interpreting the findings of our study. First, some girls (27%) already attained menarche at age 12, but only one reported a regular cycle. It was therefore not possible to correct for variation in menstrual cycle reliably.

As hormone levels and brain structure (e.g. Pletzer et al., 2010) have been shown to fluctuate with the menstrual cycle in adult women, we cannot rule out an influence of menstrual cycle on our findings. Second, the reliability of hormone level measurements decreases when the levels are closer to the detection limit (see e.g. Bay et al., 2004; Rosner et al., 2007). This may explain the lack of findings, probably more so at age 9. Third, our age range was very limited (9.0–9.6)/(11.7–13.1) at first and second assessment respectively, with 80% of participants between (9.1–9.4) and (11.8–12.4). Nevertheless, age at scanning or differences in scanning interval may have an influence on the results. However, correlations between age at scanning/scan interval and hormone levels or brain volumes at one assessment were not significant, apart from testosterone and age in girls at the second assessment, and this correlation disappeared when removing one outlier for age, suggesting that the influence of variation in age or scanning interval is very limited in this cohort. Fourth, while being relatively large for an imaging sample in twins, the cohort is small in terms of twin-modeling. Especially the LH analyses at age 9 (with a lot of children producing below the detection limit) could have been underpowered. When interpreting the findings, one should keep in mind that absence of (genetic) associations between density and hormone levels could originate from a lack of power. Finally, while all individuals were of approximately the same age, the developmental stages may well have differed between the sexes, with boys being in an earlier developmental stage as compared to the girls (Mul et al., 2001). Thus, effects may turn up later in the boys than the girls and would therefore have been left disguised at this early age in puberty.



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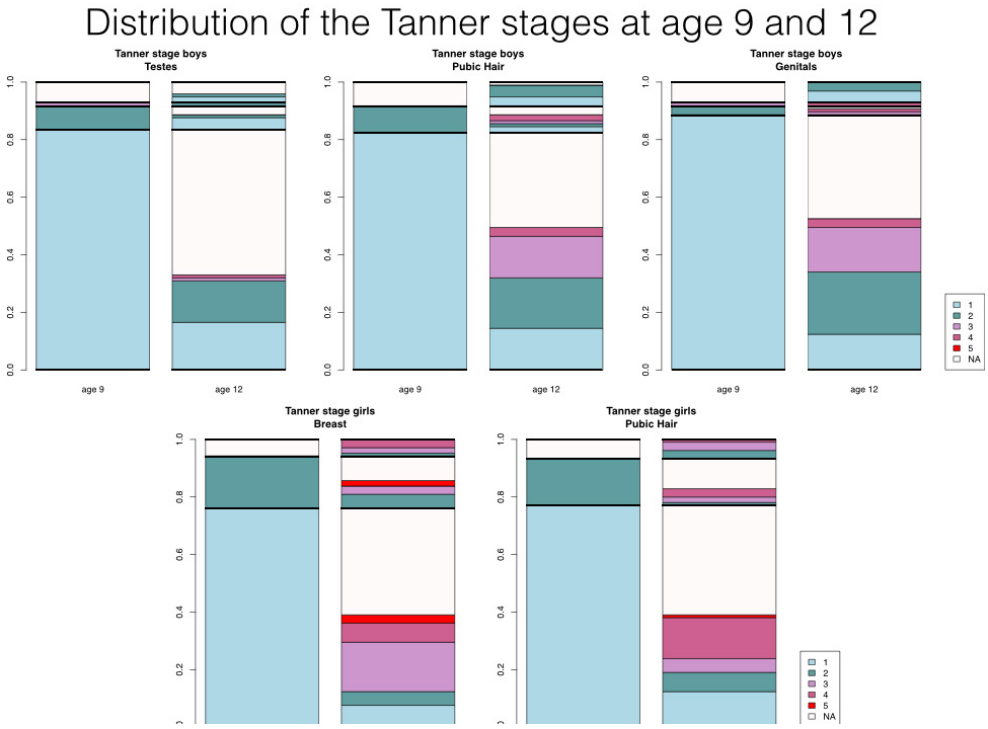
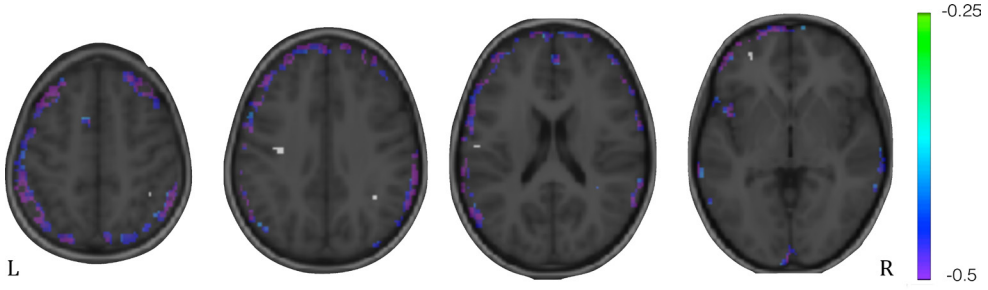
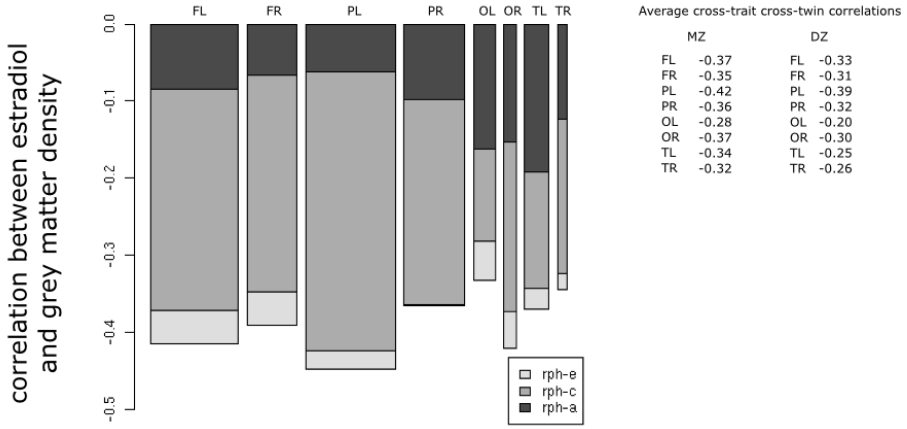
**SUPPLEMENTAL MATERIAL**




**Supplemental Figure 4.1** – Schematic overview of Tanner stages at age 9 and the transition to age 12




**Supplemental Figure 4.2** – Significant correlations between estradiol levels and grey matter density in girls, modeling age 12 only. Associations were predominantly found in frontal and parietal areas. Because this model has much less degrees of freedom, associations are much more widespread.




**Supplemental Figure 4.3** – The observed correlations (mean over significant voxels per lobe) between estradiol levels and grey matter density from the model at age 12 only split into genetic (dark grey), common environmental (grey) and unique environmental (light grey) components for each lobe. F=frontal, P=parietal, O=occipital, T=temporal, L=left, R=right. The width of the bars represent the amount of voxels that showed a significant correlation as a percentage of the number of grey matter voxels per lobe. Percentages were 21.7%; 22.4%; 5.3%; 5.9% for the left F/P/O/T lobe respectively, and 12.4%; 15.2%; 3.0% ; 2.3% for the right F/P/O/T lobe respectively. The three color bars add up to the observed correlation. F=frontal, P=parietal, O=occipital, T=temporal, L=left, R=right.

Chapter 5

# Development of the brains' structural network efficiency in early adolescence: a longitudinal DTI twin study

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**ABSTRACT**

The brain is a network and our intelligence depends in part on the efficiency of this network. The network of adolescents differs from that of adults suggesting developmental changes. However, whether the network changes over time at the individual level and, if so, how this relates to intelligence, is unresolved in adolescence. Also, the influence of genetic factors in the developing network is not known. Therefore, in a longitudinal study of 162 healthy adolescent twins and their siblings (mean age at baseline 9.9 [range 9.0-15.0] years), we mapped local and global structural network efficiency of cerebral fiber pathways (weighted with mean FA and streamline count) and assessed intelligence over a three-year interval. We find that the efficiency of the brain's structural network is highly heritable (locally up to 74%). FA-based local and global efficiency increases during early adolescence. Streamline count based local efficiency both increases and decreases, and global efficiency reorganizes to a net decrease. Local FA-based efficiency was correlated to IQ. Moreover, increases in FA-based network efficiency (global and local) and decreases in streamline count based local efficiency are related to increases in intellectual functioning. Individual changes in intelligence and local FA-based efficiency appear to go hand in hand in frontal and temporal areas. More widespread local decreases in streamline count based efficiency (frontal cingulate and occipital) are correlated with increases in intelligence. We conclude that the teenage brain is a network in progress in which individual differences in maturation relate to level of intellectual functioning.



**INTRODUCTION**

The brain is a network (Achard and Bullmore, 2007; Bullmore and Sporns, 2009; van den Heuvel and Hulshoff Pol, 2010). This network is highly significant in health and disease (Bassett and Bullmore, 2009). Structural and functional properties of the network have been related to intelligence in healthy subjects (Bassett et al., 2009; van den Heuvel et al., 2009; Langer et al., 2012; Li et al., 2009; Park and Friston, 2013; Wen et al., 2011). Structural and functional abnormalities in the network are found in neuropsychiatric illness such as schizophrenia (Fornito et al., 2012; van den Heuvel et al., 2010; van den Heuvel et al., 2013b; Hulshoff Pol and Bullmore, 2013). There are indications that developmental changes occur in the brain's network: Individual resting state network maturation (Lee et al., 2013; Smyser et al., 2010) and anatomical development (Fan et al., 2011; Nie et al., 2014) has been reported in newborns, early childhood and in early adolescence (Sherman et al., 2014). Moreover, cross-sectional studies have shown that the functional network of adolescents differs from that of adults (Fair et al., 2008) and that structural network efficiency is dependent of age (Dennis et al., 2013; Gong et al., 2009; Hagmann et al., 2010; Wen et al., 2011). A recent study describes that structural white matter development during late adolescence influences the whole network, but especially connections between hub regions (Baker et al., 2015). However, much remains unknown about this network in early adolescence.

Whether, and if so how, the structural brain network changes over time at the individual level in adolescence is not known. This is relevant since adolescence is a time characterized by rapid changes in the brain (Brouwer et al., 2012; Giedd et al., 1999; Paus, 2010; Schnack et al., 2015; van Soelen et al., 2012a; van Soelen et al., 2013; Toga et al., 2006) as well as in intelligence (Deary et al., 2012; Deary, 2012; Plomin, 2012; Waber et al., 2012) and

their interactions (Burgaleta et al., 2014a; Ramsden et al., 2011; Shaw et al., 2006). Genetic factors may play a role in development: Brain size (Peper et al., 2007; Posthuma et al., 2002; Stein et al., 2012; Thompson et al., 2001), white matter integrity (Brouwer et al., 2010; Jahanshad et al., 2013), intelligence (Bouchard and McGue, 2003; Davies et al., 2015; Plomin and Deary, 2015; van Soelen et al., 2011) and their interactions (Brouwer et al., 2014; Chiang et al., 2009; Chiang et al., 2011; Chiang et al., 2012; Posthuma et al., 2002; Stein et al., 2012) are influenced by (common) genes. This begs the questions to what extent the developing brain's network is influenced by genes and to what extent intelligence is related to the white matter network in early adolescence.

There are several ways to describe topological properties of the brain network. A structural brain network consists of anatomical locations in the gray matter (nodes) and white matter fibers connecting the nodes (edges). Mathematical representations of the structural connectivity of the human brain network have revealed that the brain is organized according to a highly efficient small-world topology combining a high level of segregation (local efficiency) with a high level of integration (global efficiency) (Achard and Bullmore, 2007). Global efficiency is a network attribute that quantifies how easy information can be exchanged over the network, providing information on the communication efficiency of a network as a whole. Local efficiency reflects how well information can travel in the direct neighborhood of a node, and is often interpreted as a metric of the local information processing capacity of a network (Bullmore and Sporns, 2012; van den Heuvel and Hulshoff Pol, 2010; Latora and Marchiori, 2001).



We mapped global and local efficiency of structural brain networks of healthy adolescents in a longitudinal, extended, twin design. Structural brain networks were based on fractional anisotropy and streamline counts from diffusion tensor imaging scans. Using structural equation modeling (Boker et al., 2011) and network connectivity analyses (Rubinov and Sporns, 2010), we estimated the heritability and development of the brain network in adolescence at a three-year interval. Associations between structural brain network topology and intelligence were investigated.

## MATERIALS AND METHODS

### *Participants*

A total of 120 twins [57 monozygotic subjects (28 boys, 29 girls), 63 dizygotic subjects (34 boys, 29 girls)] and 42 of their older siblings (17 boys, 25 girls) were included from the BrainSCALE cohort (van Soelen et al., 2012b). Zygosity of the twins was confirmed by genome-wide SNP data. At the first measurement, mean (SD) age was 9.9 (1.4) years, at the second measurement 12.9 (1.4) years, resulting in a narrow spread in age range over the interval of mean (SD) 2.92 (0.23) years. The sample was recruited from the Netherlands Twin Register (van Beijsterveldt et al., 2013). Written informed consents were obtained from all subjects and their parents. The Dutch Central Committee on Research involving Human Subjects (CCMO) approved the study.

### *Cognition*

Level of intelligence was estimated based on the intelligence quotient (IQ) as measured with the Wechsler Intelligence Scale for Children III (WISC-III, Dutch version). At the first mea-

surement, all subtasks of the WISC were included. At the second measurement, six subtasks of the WISC-III were administered: four verbal subtests (similarities, arithmetic, vocabulary, and digit span), and two non-verbal subtests (picture completion and block design). Scores on individual subtests from the WISC-III were standardized against age-specific norms, together providing a total IQ score. For two subjects, IQ values were excluded from the analyses based on a very large (absolute value > 30) change in IQ points over time. Post-hoc analyses showed that these two individuals showed no exceptional changes on the network measures (all within one standard deviation of the mean).

The shortened version of the WISC-III provided a reliable estimate of full scale IQ: at the first measurement, full scale IQ correlated highly with the IQ measurement based on the six subtasks ($r$ = 0.93 [0.90 - 0.95]). Mean (SD) IQ was 103 (14) at the first measurement (IQ based on six subtasks was 104 (15)), and 102 (16) at the second measurement. There was no significant mean change (-1.15 IQ points) over time, but considerable individual spread in IQ change (SD =10.4 IQ points change) across participants. The correlation between IQ of measurement 1 (based on 6 subtasks) and measurement 2 was 0.78 [0.70 – 0.84]. Changes in IQ were based on IQ using the six subtasks.

***MRI acquisition***

All MRI brain scans were acquired at the University Medical Center Utrecht on a 1.5 Tesla Philips Achieva scanner (Philips, Best, The Netherlands) using the same protocol at both measurements (Brouwer et al., 2012). For anatomy, a three-dimensional T1-weighted scan (Spoiled Gradient Echo; TE = 4.6 ms; TR = 30 ms; flip angle 30°; 160–180 contiguous coronal slices of 1.2 mm; in-plane resolution 1 x 1 mm2; acquisition matrix 256 x 256) of the whole head was made of each individual. For white matter fiber tracking Diffusion Tensor Images were acquired. Two Single Shot Echo Planar Imaging (SS-EPI) DWI scans were acquired (32 diffusion-weighted volumes with diffusion weighting b = 1000 s/mm2 and 32 non-collinear diffusion gradient directions; 8 diffusion-unweighted (b = 0 s/mm2) scans; TE = 88 ms; TR = 9822 ms; parallel imaging SENSE factor 2.5; flip angle 90°; 60 transverse slices of 2.5 mm, no gap, FOV 240 mm; 128 x 128 reconstruction matrix; 96 x 96 acquisition matrix, no cardiac gating) for optimal signal-to-noise ratio.

***MRI processing***

White matter pathways, referred to as fibers or tracts, were reconstructed using streamline tractography. First, the 2 DWI scans were combined and corrected for possible gradient-induced distortions (Andersson and Skare, 2002). Next, the diffusion pattern in each voxel was fitted to a tensor matrix using a robust M-estimator (Chang et al., 2005), providing three eigenvectors (representing the three principal directions of diffusion) and corresponding eigenvalues. Fractional anisotropy (FA) values were calculated in each voxel as a measure of microstructural directionality from the eigenvalues (Basser and Pierpaoli, 1996). Then, the b0 scan was registered to the T1-weighted scan using a rigid transformation (no scaling), based on optimization of a mutual information metric (Maes et al., 1997), and the T1-weighted images were nonlinearly warped into model space per measurement up to a scale of 1 mm (Collins et al., 1995), and then to the model of the second measurement. All possible fiber tracts between two regions were reconstructed in individual space using the

diffusion tensor images with an in-house implementation of the fiber assignment by continuous tracking (FACT) algorithm (Mori and Van Zijl, 2002) with 8 seed points per voxel, FA threshold of 0.1 and maximal angle of 45 degrees. The fiber tracts were warped into model space, using the concatenation of the transformations between the b0 scan and T1 scan, and between the T1 scan and model space. For network construction, the AAL template (Tzourio-Mazoyer et al., 2002) was warped onto the model brain, segmenting the cortex in a parcellation map consisting of 90 regions.

***Construction of structural brain networks***

A network consists of a set of nodes and connections (edges) that can be mathematically expressed as a graph with a collection of nodes and a collection of edges between the nodes (Bullmore and Bassett, 2011). Whole brain networks were created based on the 90 AAL brain regions. Two structural weighted brain networks were created for each individual, one for each measurement, by combining the collection of reconstructed fiber tracts with the individual parcellation maps (van den Heuvel et al., 2010; van den Heuvel et al., 2013b). Because of the longitudinal nature of our data, we decided to use a stringent threshold to ensure data quality, i.e., only those connections that were reconstructed at both ages for an individual were included in the network of that individual. Thus, network nodes *i* and *j* were defined as structurally connected by an edge when from the total collection of reconstructed streamlines at least one fiber interconnected them at both measurements. As a result, the individual topology of the networks was kept the same for both measurements. For each edge, weight $w_{ij}$ was defined by the mean FA value of the traced fibers between region *i* and *j*. We also investigated networks with streamline counts as weights to allow for a more direct comparison with studies investigating the structural network based on streamline count.



***Graph analysis***

To measure changes in efficiency, we computed global and local efficiency (Latora and Marchiori, 2001) for each individual at each measurement using the Brain Connectivity Toolbox (Rubinov and Sporns, 2010, http://www.brain-connectivity-toolbox.net). Global efficiency $E^w$ and local efficiency $E^w_{loc,i}$ were mathematically defined as:

Global efficiency:

$$E^w = \frac{1}{n}\sum_{i\in N}\frac{\sum_{j\in N, j\neq i}\left(d_{ij}^w\right)^{-1}}{n-1}$$

Local efficiency of node *i*:

$$E^w_{loc,i} = \frac{\sum_{j,h\in N, j\neq i}\left(w_{ij}w_{ih}\left[d^w_{jh}(N_i)^{-1}\right]\right)^{1/3}}{k_i(k_i-1)}$$

with $N$ the set of all nodes in the network, and $n$ the number of nodes; $k_i$ the number of edges connected to node *i*; the shortest weighted path length between *i* and *j*; and $d_{jh}(N_i)$ the length of the shortest path between *j* and *h*, in the network $N_i$ that contains only the neighbors of *i*. The symmetrical weights ($w_{ij} = w_{ji}$) were either average FA of the connection between *i* and *j* ($0 \leq w_{ij} \leq 1$), or the number of streamlines between *i* and *j* ($w_{ij} \geq 1$).

Subsequently, because of the longitudinal nature of this study, we wanted to control for the expected changes in the weights over time, while keeping the topology the same. We normalized each individual matrix by the total sum of the matrix, thereby correcting for the overall change in average FA/streamline count that could by itself already introduce a change in efficiency. The resulting matrices shed light on the redistribution of the weights over time relative to the total change of weight. For these matrices we recomputed the network measures. In addition, to compare the normalized results with the golden standard used in the literature, we also compared our networks metrics to the networks metrics of 500 random graphs that were created from each individual FA or streamline-weighted matrix. Random networks were made by randomly re-distributing the edges of the original individual network, while keeping the degree distribution the same as the original network. We reran our analyses using the metrics Eloc/Eloc$^{random}$ and Eglob/ Eglob$^{random}$. These metrics reflect how the original network is related to a network in which the weights are randomly distributed. As a result, when we look at change over time, they provide information on the redistribution of the weights relative to the each other.

Figures were created with the BrainNet Viewer (Xia et al., 2013).

***Twin modeling***

Relative influences of genetic and environmental factors were examined in an extended twin design by comparing within-pair correlations between monozygotic (MZ) and dizygotic (DZ) twins / twin sibling pairs. Differences between these correlations may arise because monozygotic twins share (almost) 100% of their genetic makeup and twin-sibling pairs, like dizygotic pairs, share on average 50% of the segregating genes (Posthuma et al., 2003). When an MZ correlation is twice as high as a DZ correlation, this indicates that a variable is largely influenced by genetic factors. In addition to genetic factors, resemblance between twins can arise from common environment, which comprises those environmental factors that induce similarity in children growing up in the same family. The presence of common environmental factors is suggested when correlations in DZ twins are larger than half the MZ correlation. When the MZ correlations are more than twice the DZ correlations, there is a suggestion for non-additive genetic influences (epistasis or dominance). Unique environmental influences are not shared with other family members and also contain the measurement error.

The same rationale as described for the univariate case can be applied to a bivariate case. If a correlation exists between two variables, the cross-trait cross-twin/sibling correlations give information whether the same genes, or environment are responsible for the association.

***Genetic analyses***

The proportion of the total variance that can be attributed to genetic or environmental factors gives estimates of (univariate) heritability ($h^2$), unique environmental influence, and common environmental influence ($c^2$) or non-additive genetic influences ($d^2$). In the latter case, we present estimates of broad heritability ($h^2 + d^2$). A genetic correlation $r_g$ was computed as the genetic covariance between two traits, divided by the square roots of the part of variances that can be attributed to genetic factors for each trait (for details see Koenis et al., 2013).

To test for longitudinal changes, individual differences over time were entered in a saturated model, in which we tested whether the mean could be constrained to zero. Based on the MZ and DZ/twin-sibling correlations, a bivariate model including additive, non-additive genetic and environmental effects was fit to the network data, incorporating age-corrected longitudinal measurements with two time points in one model. Since we had found earlier that both genes and shared environment contribute to variation in IQ in this cohort (van Soelen et al., 2011), associations between IQ (change) and (changes in) network measures were investigated in a model that allowed possible shared environmental influences on (changes in) IQ, and possible non-additive influences on (change in) network measures. All analyses were performed using structural equation modeling in OpenMx (Boker et al., 2011).

## RESULTS

### *Efficiency of the FA-weighted brain network increases during early adolescence*

Increases in both global ($p < 0.0001$) and average local network efficiency ($p < 0.0001$) were found after the three-year interval (Figure 5.1; Table 5.1). Increases in individual network efficiency occurred in a large majority (75%) of subjects and across most regions (significant after Bonferroni correction for multiple comparisons, see Figure 5.1 and Supplemental Table 5.1).

Following correction for overall FA changes that occur with age – by dividing the connectivity matrix by the sum of the matrix – there was no increase in normalized global efficiency, but the increase in average normalized local efficiency remained significant (Table 5.1). Global network efficiency of the randomized networks showed a similar increase in network efficiency over time as the individual brain networks. The increase in average local efficiency was larger in the individual brain networks than in the randomized networks (Table 5.1). Thus, while changes of FA and changes in network measures are partly overlapping, a change in FA distribution adds uniquely to (changes in) network efficiency.

### *Genetic influences on FA-weighted network characteristics*

Because of the observed age effect, in the following analyses, all network data were corrected for age. At the age of 10 years, there were significant influences of genes on global (32% [95% CI = 7-59]) and local (up to 74%; average local efficiency over the entire brain 37% [0.05-0.68]) network efficiency (Figure 5.2; Supplemental Table 5.1). MZ correlations were 0.34 [-0.21 - 0.65] for global efficiency and 0.50 [-0.13 - 0.74] for average local efficiency. DZ/twin-sib correlations were 0.04 [-0.13 - 0.24] for global efficiency and 0.02 [-0.14 - 0.20] for average local efficiency.

At second measurement, at the age of 13 years, heritability estimates were 48% [0.20 - 0.67] for global and up to 75% for local efficiency (average local efficiency 40% [14-60]). MZ correlations were 0.43 [0.09 - 0.66] for global efficiency and 0.35 [0.03 - 0.59] for average local efficiency. DZ/twin-sib correlations were 0.15 [-0.08 - 0.38] for global efficiency and 0.08 [-0.13-0.30] for average local efficiency. Over time, a stable genetic factor influenced global (genetic correlation $r_g = 1.00$ [0.63 - 1.00]) and average local ($r_g = 1.00$ [0.53 - 1.00]) efficiency.

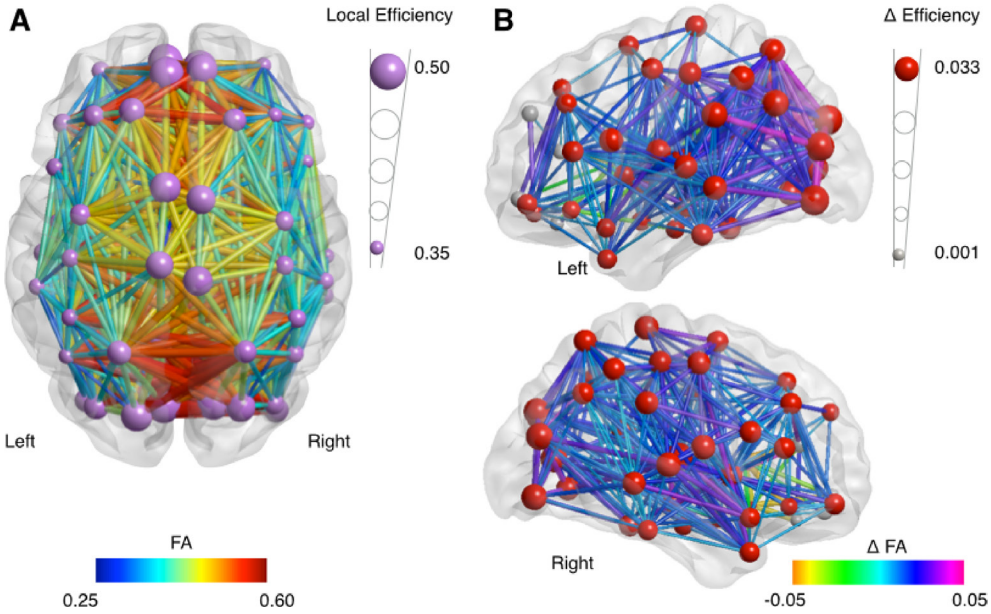


**Figure 5.1** – Development of FA-based network efficiency. (A) Efficiency of the structural brain network at age 10. Larger nodes represent a higher level of efficiency. Edges in the network are colored by fractional anisotropy (FA, a measure for white matter integrity); higher values in red, lower values in blue. For details on local efficiency at measurement 1 and measurement 2, see Supplemental Table 5.1. (B) Development of the structural brain network over a three-year interval with significant change Δ in local network efficiency shown in red nodes ($p < 0.05/90$; larger nodes represent a larger increase), and significant change in edges colored for FA change (for visualization purposes only edges with a significant change at $p < 0.005$ are shown). See Supplemental Table 5.1 for details.

**Table 5.1** – Longitudinal changes in FA-weighted global and local network efficiency measures.

| | Measurement 1 | Measurement 2 | Change | %Change |
|---|---|---|---|---|
| Age | 9.9 (1.4) | 12.9 (1.4) | 2.92 (0.23) | |
| IQ | 103 (14) | 102 (16) | -1.15 (10.4)[a] | -1.2% |
| FA | 0.450 (0.023) | 0.466 (0.022) | 0.017 (0.024)* | 3.8% |
| Global Efficiency | 0.276 (0.014) | 0.286 (0.014) | 0.010 (0.014)* | 3.6% |
| Local Efficiency[b] | 0.410 (0.020) | 0.425 (0.020) | 0.016 (0.022)* | 3.9% |
| *Eglob* normalized | 3.163 (0.10) (x10$^{-4}$) | 3.161 (0.11) (x10$^{-4}$) | -0.001 (0.010) (x10$^{-4}$) | 0.0% |
| *Eloc* normalized | 4.711 (0.24) (x10$^{-4}$) | 4.717 (0.24) (x10$^{-4}$) | 0.006 (0.009) (x10$^{-4}$)* | 0.1% |
| *Eglob/Eglob$^{random}$* | 0.922 (0.009) | 0.923 (0.009) | 0.000 (0.004) | 0.1% |
| *Eloc/Eloc$^{random}$* | 1.125 (0.022) | 1.126 (0.022) | 0.001 (0.002)* | 0.1% |

Means (standard deviations) and percentage change (%); FA represents the average fractional anisotropy of the structural brain network.

[a] When looking at the change scores based on 6 subtests at both assessments, IQ decreased with 1.71 points.

[b] Local efficiency as averaged over the entire brain.

*Significant changes ($p < 0.05$)

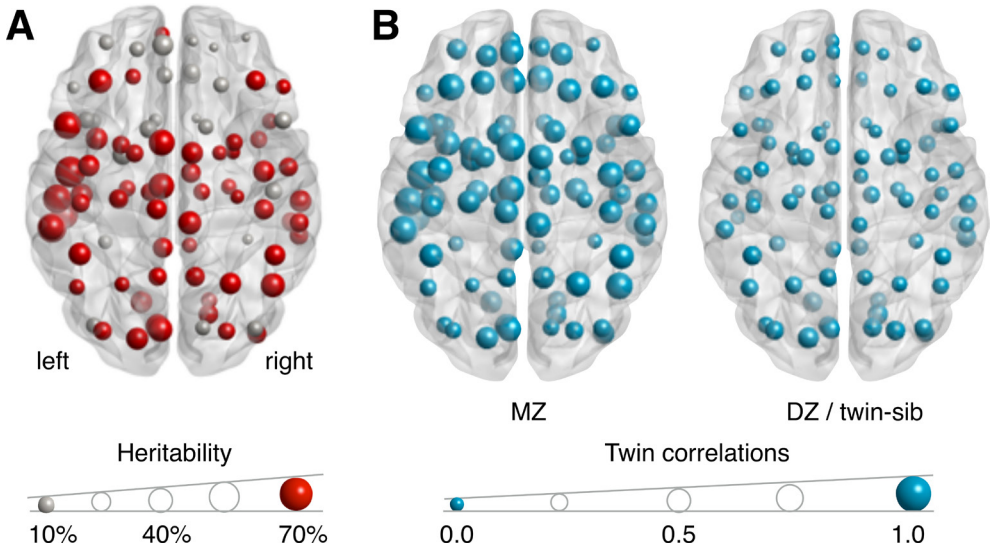


**Figure 5.2** – (A) Significant heritability (up to 74%) of local efficiency at age 10. Size of the nodes represents the heritability of local efficiency. See Supplemental Table 5.1 for a complete overview of the heritability of local efficiency at first and second measurement. (B) Estimated correlations between members of a twin pair for local efficiency for monozygotic (MZ) twin (left) and dizygotic (DZ) twin / twin-sibling pairs (right), with MZ correlations ranging up to 0.75.



***(Genetic) Associations of FA-weighted network efficiency with intelligence***

A higher average local efficiency was associated with a higher level of intellectual functioning, significantly at age 13 (first measurement M1: $r$ = 0.13 [-0.04 - 0.29]; $p$ = 0.12; second measurement M2: $r$ = 0.16 [0.01 - 0.32]; $p$ = 0.047). This association appeared to be driven by performance IQ (M1: $r$ = 0.17; p = 0.047; M2: $r$ = 0.19; p = 0.039) rather than verbal IQ (M1: $r$ = 0.07; p = 0.39; M2: $r$ = 0.09; $p$ = 0.29). At age 13, the association between average local efficiency and IQ was caused by shared genes influencing by both phenotypes (genetic correlation $r_g$ = 1.00 [0.13 - 1.00]; $p$ = 0.016). There were no associations between global efficiency and intelligence.

At a nodal level, at both measurements the association between local efficiency and IQ was particularly prominent in frontal and temporal areas; e.g., in the inferior frontal cortex, insula, superior temporal pole, superior and middle temporal gyri, Heschl's gyrus, angular gyrus, bilaterally although most prominently on the right side (Figure 5.3; Table 5.2). These associations could for a large part be explained by genetic factors influencing both intelligence and network efficiency (Figure 5.3; Table 5.2).

***Changes in FA-weighted network efficiency are related to changes in intelligence***

When assessing changes in network efficiency and changes in intelligence within individuals over time, adolescents who showed the most prominent increase in efficiency of the structural brain network were the ones who gained (most) in intelligence. In contrast, individuals showing a reduction - or no change - in global network efficiency, displayed a decrease in IQ ($r$ = 0.17, $p$ = 0.030) and performance IQ ($r$ = 0.18, $p$ = 0.023) but not with verbal IQ ($r$ =

0.05, $p = 0.53$). Average local efficiency change correlated with change in total IQ ($r = 0.17$, $p = 0.034$) and performance IQ ($r = 0.16$, $p = 0.045$), but not with change in verbal IQ ($r = 0.07$, $p = 0.38$). We could not disentangle whether these associations were driven by genetic and/or environmental factors.

Changes in local efficiency and concomitant change in intelligence were most evident in frontal and temporal areas (Figure 5.4; Table 5.2). Specifically, change in local network efficiency in the left inferior orbitofrontal cortex and left anterior cingulum explained over 6 percent of individual change in intelligence. The association between change in intelligence and that in local efficiency was also present in other (including right-sided) frontal areas, as well as in the superior temporal poles, insula, thalamus, putamen, pallidum and caudate nucleus.

Post-hoc analyses revealed no significant differences between boys and girls in (change of) global FA-weighted and local FA-weighted network measures, or interactions between IQ and network measures at baseline, at follow up, and over time.



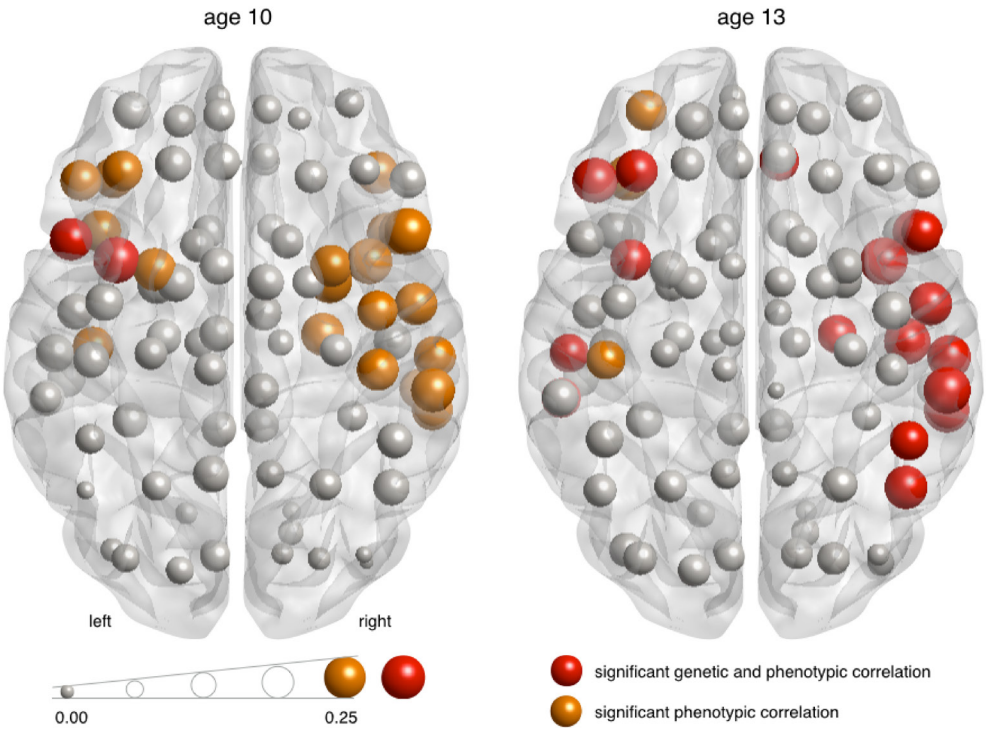


**Figure 5.3** – Associations between FA-based local efficiency and IQ. Local correlations between IQ and network efficiency range up to 0.25. Red spheres represent significant correlations between network efficiency and IQ that can be attributed to a shared genetic component. Orange spheres represent significant correlations between network efficiency and IQ for which the contributions of genes and environment could not be disentangled. The size of the spheres represents the size of the correlation. See Table 5.2 for a complete overview of regional correlations between FA-based local efficiency and IQ.

**Table 5.2** – Correlations between FA-weighted local efficiency and IQ at measurement 1 (M1) and 2 (M2), and between change in local efficiency and change in IQ

| | Left | | | Right | | |
|---|---|---|---|---|---|---|
| Region | M 1 | M 2 | Change | M 1 | M 2 | Change |
| Precentral | 0.16 | 0.13 | **0.18** | **0.18** | 0.16* | **0.17** |
| Frontal_Sup | 0.15 | 0.12 | **0.21** | 0.11 | 0.13 | **0.18** |
| Frontal_Sup_Orb | 0.11 | 0.16* | **0.22** | 0.02 | 0.12* | **0.17** |
| Frontal_Mid | **0.18** | **0.20*** | **0.20** | 0.09 | 0.10 | 0.11 |
| Frontal_Mid_Orb | 0.15 | **0.18** | **0.21** | 0.07 | 0.14* | 0.10 |
| Frontal_Inf_Oper | **0.20*** | 0.16 | **0.18** | **0.25** | **0.19*** | **0.18** |
| Frontal_Inf_Tri | **0.18** | **0.23*** | **0.20** | 0.15 | 0.14 | 0.12 |
| Frontal_Inf_Orb | **0.19** | **0.23** | **0.24** | **0.20** | 0.16* | 0.14 |
| Rolandic_Oper | 0.14 | 0.12 | **0.18** | **0.20** | **0.21*** | 0.15 |
| Supp_Motor_Area | 0.15 | 0.05 | **0.17** | 0.15 | 0.09 | 0.15 |
| Olfactory | 0.11 | 0.13* | 0.15 | 0.08 | 0.15 | **0.18** |
| Frontal_Sup_Medial | 0.11 | 0.10 | **0.20** | 0.08 | 0.10 | **0.16** |
| Frontal_Med_Orb | 0.05 | 0.06 | **0.19** | 0.02 | 0.05 | **0.19** |
| Rectus | 0.06 | 0.12* | 0.12 | 0.08* | **0.17*** | **0.18** |
| Insula | **0.24*** | **0.19*** | **0.22** | **0.23** | **0.25*** | **0.19** |
| Cingulum_Ant | 0.15 | 0.14* | **0.22** | 0.06 | 0.12* | **0.20** |
| Cingulum_Mid | 0.14 | 0.04 | 0.10 | 0.14* | 0.06 | 0.13 |
| Cingulum_Post | 0.14 | 0.12* | 0.05 | 0.12 | 0.12 | 0.08 |
| Hippocampus | 0.11 | 0.07 | 0.08 | 0.11 | 0.12* | 0.11 |
| ParaHippocampal | 0.13 | 0.10 | **0.20** | **0.21** | **0.17*** | **0.16** |
| Amygdala | 0.16 | 0.11 | 0.14 | **0.23** | 0.14 | **0.16** |
| Calcarine | 0.01 | 0.05 | 0.03 | 0.04 | 0.07 | 0.08 |
| Cuneus | 0.10 | 0.09 | 0.06 | 0.00 | 0.10 | 0.08 |
| Lingual | 0.01 | 0.07 | 0.04 | -0.02 | 0.00 | 0.10 |
| Occipital_Sup | 0.06 | 0.11 | 0.02 | 0.02 | 0.11 | 0.08 |
| Occipital_Mid | 0.07 | 0.12* | 0.03 | -0.05 | 0.00 | 0.10 |
| Occipital_Inf | 0.02 | 0.13 | 0.00 | -0.07 | 0.05 | 0.12 |
| Fusiform | 0.13 | 0.15* | -0.03 | 0.09 | 0.08 | 0.10 |
| Postcentral | 0.14 | 0.17 | 0.13 | **0.20** | 0.11 | 0.14 |
| Parietal_Sup | 0.08 | 0.11 | 0.01 | 0.10 | 0.13 | 0.11 |
| Parietal_Inf | 0.08 | 0.16* | 0.06 | 0.14 | **0.17*** | **0.19** |
| SupraMarginal | 0.09 | 0.14 | 0.09 | **0.22** | **0.24*** | **0.17** |
| Angular | -0.03 | 0.11 | 0.02 | 0.14 | **0.21*** | 0.15 |
| Precuneus | 0.15 | 0.09 | 0.04 | 0.06 | 0.04 | 0.10 |
| Paracentral_Lobule | 0.10 | 0.04 | 0.12 | 0.13 | -0.04 | -0.03 |
| Caudate | 0.12 | 0.10 | **0.19** | 0.12 | 0.10* | **0.18** |
| Putamen | **0.20** | 0.16* | **0.19** | **0.20** | 0.14* | **0.20** |
| Pallidum | 0.16 | 0.11* | **0.17** | 0.11 | 0.12 | **0.17** |
| Thalamus | 0.08 | 0.05 | **0.19** | 0.05 | 0.09 | **0.20** |
| Heschl | **0.18** | 0.14* | **0.16** | 0.16 | **0.24*** | 0.15 |
| Temporal_Sup | 0.16 | **0.17*** | **0.18** | **0.20** | **0.21*** | **0.17** |
| Temporal_Pole_Sup | **0.19** | 0.15* | **0.21** | **0.22** | **0.23*** | **0.21** |
| Temporal_Mid | 0.13 | **0.17*** | **0.20** | **0.19** | **0.23*** | 0.13 |
| Temporal_Pole_Mid | 0.11 | 0.15* | **0.18** | **0.18** | 0.16* | **0.18** |
| Temporal_Inf | 0.16 | 0.14 | 0.12 | **0.18** | 0.12* | 0.13 |

Bold: significant phenotypic correlations ($P < 0.05$)

* Observed correlation explained by a significant genetic association

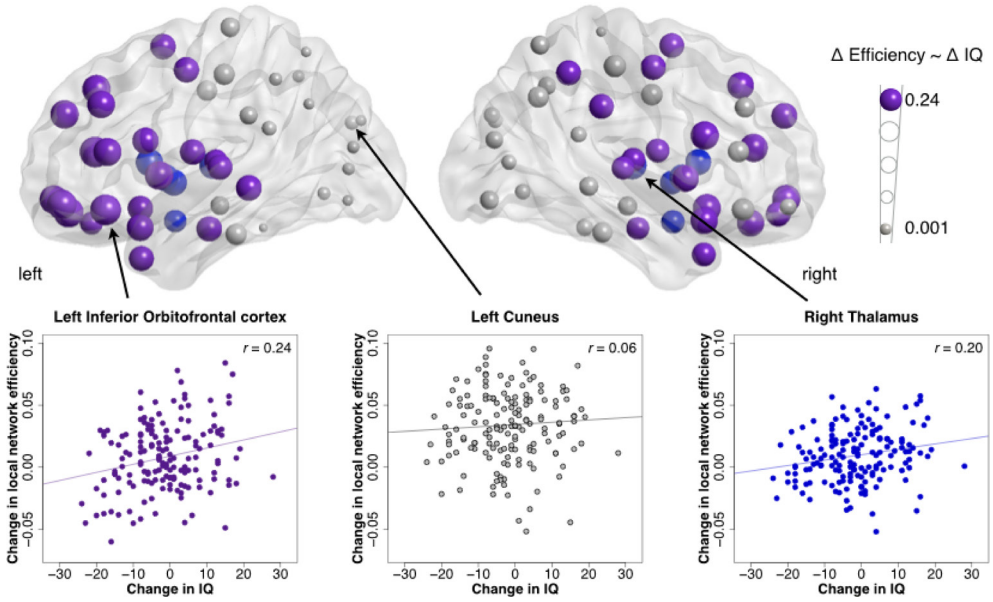


**Figure 5.4** – Development of network efficiency and intelligence in adolescence. Correlations between the change in local efficiency and change in IQ [nodes with a significant correlation are colored in purple (cortical) and blue (subcortical)]; with examples of scatterplots of correlation of local efficiency change in left orbitofrontal cortex (significant; left panel), left cuneus (non-significant; middle panel), and right thalamus (significant; right panel) with change in IQ. See Table 5.2 for a complete overview of regional correlations between changes FA-based local efficiency and changes IQ.

***Analyses on networks weighted with streamline count***

Streamline count based network efficiency was higher in boys at both measurements (see Supplemental Table 5.2). Because of this sex effect in the network characteristics when weighing the network with streamline count, the analyses on streamline count weighted networks were corrected for sex beforehand. This did not change the results on change over time, associations with intelligence or the genetic modeling results.

***Efficiency of the streamline count weighted brain network increases and decreases during early adolescence***

Both global ($p < 0.001$) and average local ($p = 0.012$) efficiency decreased in the three year follow up period. Locally, efficiency increased in frontal and occipital areas, and decreased in subcortical, temporal and parietal regions (Figure 5.5; Supplemental Table 5.2).

Compared to random networks, average local efficiency slightly decreased ($p = 0.045$) but global efficiency did not change. When we normalized the matrices, global efficiency decreased ($p = 0.009$) but normalized average local efficiency did not change.

***Genetic influences on streamline count weighted network characteristics***

Because of the observed age and sex effects, in the following analyses, all data were corrected for age and sex beforehand.

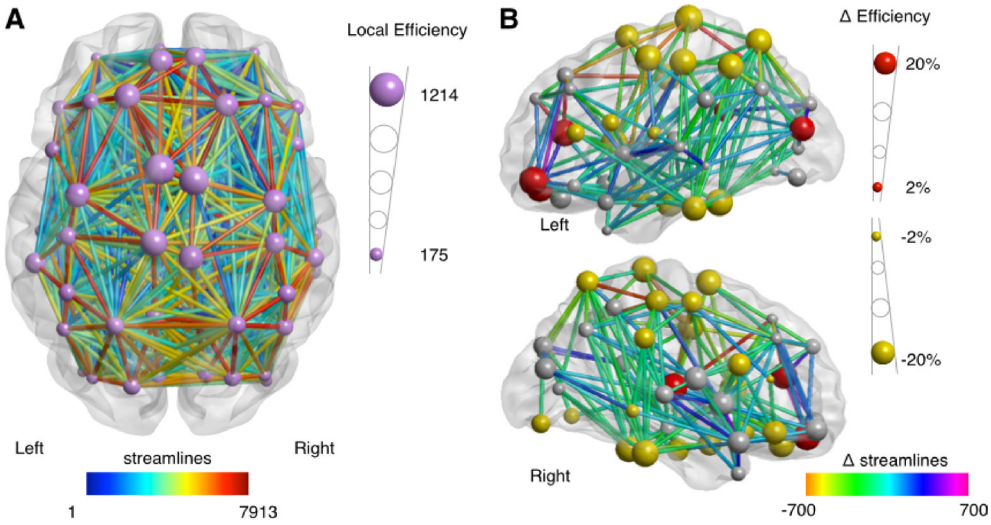


**Figure 5.5** – Development of streamline count based network efficiency. (A) Efficiency of the structural brain network at age 10. Larger nodes represent a higher level of efficiency. Edges in the network are colored by number of streamlines; higher values in red, lower values in blue. For details on local efficiency at measurement 1 and measurement 2, see Supplemental Table 5.2. (B) Development of the structural brain network over a three-year interval with significant ($p < 0.05/90$) increase in local network efficiency shown in red nodes and significant decreases are shown in yellow nodes; larger nodes represent a larger absolute change. Edges are colored by significant changes in streamline counts. For visualization purposes, only edges with a significant at $p < 0.005$ change are shown. See Supplemental Table 5.2 for details.



Local and global efficiency of streamline count weighted networks were also highly heritable at both measurements with heritability for local efficiency up to 64% (Supplemental Table 5.3). Heritability estimates were: Average local efficiency 37% [12 – 65], global efficiency 60% [27 - 78] for M1; average local efficiency 24% [6 –47]), global efficiency 24% [6 – 45] for M2. Over time, a stable genetic factor influenced global ($r_g$ = 0.87 [0.37 - 1.00]) and average local ($r_g$ = 0.96 [0.19 - 1.00]) efficiency. In addition, a number of regions showed genetic influences on the change in local efficiency (Supplemental Table 5.3).

***Changes in streamline count weighted network efficiency are related to changes in intelligence***

We did not find a relation between network efficiency and IQ scores at age 10 or 13. Nevertheless, a change in efficiency was correlated to a change in IQ: individuals who gained in IQ scores showed a decrease in average local efficiency ($r$ = -0.16, $p$ = 0.043), which was driven by performance IQ (r = -0.22, p = 0.005) rather than verbal IQ (r = 0.02, p=0.79). Genetic and/or environmental correlations were not significant. A similar pattern was found for global efficiency, but only the association with performance IQ reached significance (r = -0.20, p = 0.010) but again, influences of genetic and environmental correlations could not be disentangled. Local regions with largest relations were left inferior orbitofrontal cortex and left caudate, followed by bilateral anterior cingulum and left middle occipital gyrus

(Supplemental Table 5.4). IQ reached significance (r = -0.20, p = 0.010) but again, influences of genetic and environmental correlations could not be disentangled. Local regions with largest relations were left inferior orbitofrontal cortex and left caudate, followed by bilateral anterior cingulum and left middle occipital gyrus (Supplemental Table 5.4).

**DISCUSSION**

Here we show in a longitudinal study in 162 young adolescent twins and their siblings, that the efficiency of the brain's structural network increases during early adolescence. We are one of the first studies to measure structural network development in adolescence using a longitudinal approach. Both increases in white matter integrity (fractional anisotropy; FA) and reorganization of the network contribute to an increase in FA-based global and local efficiency. At the same time, local increases and decreases in streamline count and a reorganization of the network both contribute to a net decrease in streamline count-based network global efficiency. Moreover, these changes in network efficiency are related to intelligence; changes in intelligence and in the structural brain network efficiency appear to go hand in hand. This effect seemed stronger in FA based efficiency, which showed a clear pattern of frontal and temporal increases of local efficiency that were related to an increase IQ. To a smaller extent, streamline count based local efficiency was negatively related to changes in IQ in a more widespread pattern in frontal, cingulate and occipital regions.



We report moderate to high heritability for efficiency of the brain's structural network (locally up to 74% for FA-based networks; up to 64% for streamline count based networks) in early adolescence. For both average local and global efficiency, the genetic factor influencing the efficiency measure remained stable during development in adolescence. Besides, the relation between IQ and FA-based local network efficiency was completely explained by genes shared by both phenotypes. Our findings extend those from cross-sectional studies in adults that report genetic effects on structural network topology (Bohlken et al., 2014; Jahanshad et al., 2012) and on functional network topology in adults (Fornito et al., 2011) and in this cohort (van den Heuvel et al., 2013a). This might imply that over time the genetic influence on local structural network organization remains stable.

A higher intelligence was accompanied by a more local efficient fiber integrity (FA) based network, but not related to streamline-based local or global efficiency. These associations (up to 0.25) are of comparable magnitude as the (genetic) association between intelligence and whole brain volume in adults (Posthuma et al., 2002). The positive associations between IQ and FA-based local efficiency were particularly prominent in frontal and temporal nodes, emphasizing the relevance of frontal and temporal regions for intelligence (Haier et al., 1988; Haier et al., 2004), in accordance with the P-FIT regions (parieto-frontal integration theory of intelligence) regions (Jung and Haier, 2007). Moreover, it emphasizes that how these regions are connected with the rest of the brain is important. This extends prior reports on positive associations between intelligence and global and local structural network efficiency in adults (Li et al., 2009; Wen et al., 2011) by showing that the two are genetically related, already in childhood. This result relates to previous findings that local efficiency of the functional network in frontal, parietal and temporal regions is related to IQ in children and adolescents (Wu et al., 2013) and in adults (van den Heuvel et al., 2009; Langer et al., 2012; Santarnecchi et al., 2014). Indeed, gray matter regions associated with

intelligence based on volumetric measures were more densely connected than on average, which underlines the importance of connectivity between cortical regions implicated in intelligence (Bohlken et al., 2016). In addition, other studies have reported that the strength of functional connectivity between the prefrontal cortex and the rest of the brain is related to IQ (Cole et al., 2012; Song et al., 2008), and suggested that enlargement of the rostral putamen is related to IQ through its functional relation with the inferior parietal cortex and insula (Burgaleta et al., 2014b). However, despite the visual overlap of our results (Figure 5.3) with previous mentioned regions that are involved in intellectual functioning, we did not find direct evidence for P-FIT regions to be overly represented in our findings: 6 or 8 significant regions out of 24 P-FIT regions versus 17 or 12 significant regions out of 66 non-P-FIT regions (chi square tests p=0.94 and p=0.21 at measurement 1 and 2, respectively). Nonetheless, as the participants in this study were rather young, it could be that at an older age, correlations with IQ become more crystalized in the brain. To date there are only two studies that describe a correlation between DTI network efficiency (global and local) and IQ; one study in young adults between 17 and 33 years (Li et al., 2009) and one in older people between 72 and 90 years (Wen et al., 2011). In addition, it is likely that the structural brain associations with IQ are not stable throughout life; for instance, cortical thickness develops differently depending on IQ (Brans et al., 2010; Brouwer et al., 2014; Karama et al., 2009; Schnack et al., 2015; Shaw et al., 2006). Thus, possibly, the association between local efficiency and IQ grows with network maturation when also the relation between global efficiency and IQ becomes evident.



Importantly, the subjects with most prominent maturational changes in brain wiring via FA or streamline count also showed a positive change in IQ. Although intelligence was stable over the three-year follow-up in the majority of individuals in our cohort, one in six adolescents showed a substantial change in their IQ score (> 15 points). Such considerable changes in IQ scores have been reported in other adolescent cohorts, implicating that an individual's intellectual capacity relative to their peers can decrease or increase during adolescence (Burgaleta et al., 2014a; Ramsden et al., 2011; Waber et al., 2012). Our findings support the existence of individual variations in long-term modification of the structural network for functional demands (Park and Friston, 2013). A relationship between change in intelligence and change in brain structure during adolescence has been shown earlier, where increases in IQ scores have been related to local increases in gray matter density (Ramsden et al., 2011) and rate of cortical thinning (Burgaleta et al., 2014a), suggesting, as we do, that individual development of intellectual capacity goes hand in hand with changes in anatomically distant brain regions. Our finding suggests that during this time of rapid intellectual development, plasticity of the brain's network is an important contributing, if not necessary, factor to maintain and possibly gain in intellectual capacity.

Over the three-year interval, we find an increase in FA and FA-based efficiency in almost all brain areas. This longitudinal finding coincides with cross-sectional studies reporting maturation of white matter integrity (Baker et al., 2015; Kochunov et al., 2012; Lebel and Beaulieu, 2011; Schmithorst and Yuan, 2010), although local decreases in FA have also been found (Baker et al., 2015). Overall increases in FA cannot solely explain our network findings, since local efficiency also increases when we correct for overall increase in FA, suggesting a redistribution of the weights in the structural network during development. In

addition, we know from previous work in adults that different genetic factors independently influence FA and network topology (Bohlken et al., 2014), which suggests that network topology provides supplementary information to FA. When we repeated our analyses using streamline count weighted networks, we find a net decrease in global efficiency, with local decreases in subcortical, temporal and parietal areas, and increases in frontal and occipital areas. This is consistent with a recent longitudinal study that found increases and decreases in streamline density in late adolescence (Baker et al., 2015). Our findings extend reports that both increases and decreases in local efficiency occur between 12 and 30 years (Dennis et al., 2013), and in adulthood (Gong et al., 2009). Other studies found an increase in global efficiency over the ages 2 to 18 (Hagmann et al., 2010) and a decrease in local and global efficiency between the ages 4 and 40 (Lim et al., 2015). Thus, considerable developmental changes take place during childhood and adolescence, not only over a broad age range, but as the current study shows also within the small age range of 3 years. Indeed, the longitudinal aspect provided statistical power to detect processes that take place during a period of rapid changes in the brain and intelligence.

When comparing the results of FA weighted with streamline count weighted networks in our cohort, the results clearly show that these measures capture different aspects of brain development. Indeed, our findings seem to imply that during development of the structural brain network, local information processing capacity improves via an increased speed of information transfer along the axons, while certain fiber bundles become more compact in volume, as was measured with net decrease in streamline count. This finding was also reflected by the differential relation between efficiency and IQ in FA versus streamline-weighted networks. FA-weighted but not streamline-weighted local efficiency correlated locally with IQ, although a change in both local streamline-weighted local efficiency and local and global FA-weighted efficiency was correlated to change in IQ. That different aspects of white matter bundles (FA, T1, MTR) can independently associate with IQ has been shown (Penke et al., 2012; Bohlken et al, unpublished data), supporting our findings. In addition, in a recent longitudinal study in late adolescence it was shown that with development both streamline and FA weights show local increases and decreases throughout brain, and there seems to be a bias towards FA-increase in hub-to-hub connections whereas streamline count shows both increases and decreases in these connections (Baker et al., 2015). Two other studies in adults performed streamline and FA based analyses and found comparable results for associations with age (Stadlbauer et al., 2012) and IQ (Li et al., 2009), thus contrasting our findings. However, possibly, the relation between FA and streamline count – and their associations with IQ – changes with brain maturation.

Because our focus was on the longitudinal aspect of our data, we used a very stringent edge selection: only those edges that could be measured twice (once at each measurement) within an individual were included in the final individual network. Using this approach, individual networks were sparser than when all traced bundles would have been included. However, we ensured a higher signal to noise ratio. When applying a mask that includes bundles present in 60% of the participants at both measurements 1 and 2 (de Reus and van den Heuvel, 2013) we find similar results, although the associations between network measures and IQ become somewhat stronger. This indicates that our stringent edge-selection did not drive our findings.

In the streamline based networks there was some discrepancy between the normalized and randomized results with regard to the correlation between change in IQ and change in local and global efficiency. This may be explained by the longitudinal setup of our study. For FA, there was an almost brain-wide increase, which we wanted to take into account. For streamline count, at a regional level both increases and decreases were found. A correction for overall change in streamline count thus includes both positive and negative changes, and that will influence the whole brain network independently of the strong local differences in maturation pattern. Comparing the individual streamline-based networks to randomized networks provides in this case a better approach because networks are compared to their own null-model with the same weight distribution.

Several studies found sex related differences in brain characteristics in children and adolescents (Ingalhalikar et al., 2014; Lebel and Beaulieu, 2011; Schmithorst and Yuan, 2010; Wu et al., 2013) and adults (Lebel et al., 2012; Tang et al., 2010; Yan et al., 2011). We found that streamline-weighted network efficiency was significantly higher in boys at baseline and follow-up, but boys and girls did not differ on change measures. In contrast, we found no sex differences in FA-weighted network measures. Since the participants in the current study are still young (mean age at baseline 9.9 years), sex differences in structural network properties could develop at a later age. There are suggestions that males and females may also have differential regional associations with intelligence (e.g. Haier et al., 2005; Tang et al., 2010; see also review by Deary et al., 2010). Although we cannot exclude that insufficient statistical power disguised differences between boys and girls, our data did not show (regional) sex-dependent longitudinal network changes or correlations between local structural efficiency and IQ at this age.

In conclusion, in this longitudinal study we show that the FA-based topological properties of the young and healthy teenage brain become more efficient with age. The streamline-based network is reorganized to a topology with decreased global efficiency via increases and decreases in local efficiency. This indicates that FA and streamline count cover different aspects of the developing brain and that maturation is not always accompanied by increases in local information processing. Moreover, the increase in FA-based local and global efficiency is related to increases in IQ, whereas in streamline-based networks, local decreases in efficiency were related to increases in IQ. This suggests that a decrease in local information processing capabilities is not per se undesirable. We also found that variation in the topology of both FA and streamline-based networks of young adolescents is partly due to genetic variation, and that genes shaping FA-based connectivity organization also benefit intelligence. Clearly, the teenage brain is a network in progress in which individual differences in network maturation relate to the level of intellectual functioning.



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## SUPPLEMENTAL MATERIAL

**Supplemental Table 5.1** – FA-weighted local efficiency and the broad heritability of local efficiency at measurement 1 (M1) and measurement 2 (M2).

| | Local efficiency | | | | Broad heritability of local efficiency | | | |
|---|---|---|---|---|---|---|---|---|
| | Left | | Right | | Left | | Right | |
| Region | M 1 | M 2 | M 1 | M 2 | M 1 | M 2 | M 1 | M 2 |
| Precentral | 0.41 | 0.42** | 0.40 | 0.42** | **0.43** | **0.24** | **0.44** | **0.48** |
| Frontal_Sup | 0.43 | 0.44** | 0.42 | 0.43** | **0.25** | **0.39** | 0.26 | **0.29** |
| Frontal_Sup_Orb | 0.41 | 0.42** | 0.42 | 0.42* | 0.25 | **0.41** | 0.03 | 0.22 |
| Frontal_Mid | 0.41 | 0.42** | 0.38 | 0.39** | **0.52** | **0.37** | **0.38** | **0.25** |
| Frontal_Mid_Orb | 0.39 | 0.41** | 0.38 | 0.39** | 0.23 | 0.16 | 0.01 | 0.08 |
| Frontal_Inf_Oper | 0.40 | 0.42** | 0.37 | 0.39** | **0.64** | **0.38** | 0.31 | **0.33** |
| Frontal_Inf_Tri | 0.40 | 0.41** | 0.38 | 0.39** | 0.14 | 0.20 | **0.23** | **0.34** |
| Frontal_Inf_Orb | 0.38 | 0.39** | 0.37 | 0.37* | 0.04 | 0.20 | 0.06 | 0.16 |
| Rolandic_Oper | 0.39 | 0.41** | 0.36 | 0.38** | **0.74** | **0.32** | **0.35** | **0.49** |
| Supp_Motor_Area | 0.46 | 0.47** | 0.45 | 0.46** | **0.59** | **0.47** | **0.45** | **0.45** |
| Olfactory | 0.39 | 0.39 | 0.39 | 0.38** | 0.17 | **0.37** | 0.03 | **0.29** |
| Frontal_Sup_Media | 0.47 | 0.48* | 0.46 | 0.46* | 0.43 | 0.16 | 0.06 | 0.19 |
| Frontal_Med_Orb | 0.48 | 0.48 | 0.47 | 0.47 | 0.22 | **0.26** | 0.13 | 0.16 |
| Rectus | 0.41 | 0.41 | 0.42 | 0.41 | 0.09 | 0.20 | 0.33 | 0.31 |
| Insula | 0.38 | 0.40** | 0.37 | 0.39** | **0.46** | **0.40** | **0.30** | **0.38** |
| Cingulum_Ant | 0.47 | 0.48 | 0.48 | 0.48 | 0.27 | 0.18 | 0.27 | **0.30** |
| Cingulum_Mid | 0.45 | 0.46** | 0.45 | 0.46** | **0.58** | **0.45** | **0.38** | **0.29** |
| Cingulum_Post | 0.47 | 0.50** | 0.48 | 0.50** | **0.39** | **0.30** | **0.23** | **0.46** |
| Hippocampus | 0.39 | 0.41** | 0.41 | 0.42** | **0.37** | **0.42** | **0.19** | **0.47** |
| ParaHippocampal | 0.35 | 0.36** | 0.36 | 0.37** | 0.39 | **0.40** | **0.21** | **0.57** |
| Amygdala | 0.36 | 0.37** | 0.36 | 0.37** | **0.54** | **0.47** | **0.38** | **0.33** |
| Calcarine | 0.46 | 0.49** | 0.46 | 0.49** | **0.53** | **0.50** | 0.23 | 0.20 |
| Cuneus | 0.48 | 0.51** | 0.47 | 0.49** | **0.46** | **0.35** | 0.27 | **0.33** |
| Lingual | 0.43 | 0.45** | 0.43 | 0.45** | **0.46** | **0.48** | **0.30** | **0.26** |
| Occipital_Sup | 0.46 | 0.49** | 0.45 | 0.48** | **0.34** | **0.37** | 0.26 | 0.14 |
| Occipital_Mid | 0.43 | 0.46** | 0.41 | 0.43** | 0.26 | 0.35 | **0.36** | 0.21 |
| Occipital_Inf | 0.42 | 0.45** | 0.43 | 0.45** | 0.20 | **0.31** | 0.09 | 0.27 |
| Fusiform | 0.38 | 0.40** | 0.39 | 0.41** | **0.34** | **0.31** | **0.39** | **0.43** |
| Postcentral | 0.39 | 0.41** | 0.40 | 0.42** | **0.22** | **0.43** | **0.44** | **0.34** |
| Parietal_Sup | 0.42 | 0.45** | 0.42 | 0.43** | **0.48** | **0.43** | **0.42** | **0.47** |
| Parietal_Inf | 0.39 | 0.41** | 0.40 | 0.41** | **0.56** | **0.22** | **0.35** | **0.35** |
| SupraMarginal | 0.38 | 0.41** | 0.38 | 0.40** | **0.25** | **0.35** | **0.53** | **0.50** |
| Angular | 0.38 | 0.41** | 0.39 | 0.41** | **0.33** | **0.46** | **0.37** | **0.34** |
| Precuneus | 0.43 | 0.46** | 0.45 | 0.47** | **0.40** | **0.41** | **0.50** | **0.49** |
| Paracentral_Lobule | 0.44 | 0.46** | 0.45 | 0.47** | 0.31 | 0.26 | 0.24 | **0.33** |
| Caudate | 0.39 | 0.39 | 0.39 | 0.39 | **0.32** | **0.31** | **0.30** | **0.45** |
| Putamen | 0.40 | 0.41** | 0.39 | 0.40** | **0.32** | **0.27** | **0.22** | **0.33** |
| Pallidum | 0.41 | 0.43** | 0.41 | 0.42** | **0.31** | 0.14 | **0.28** | 0.19 |
| Thalamus | 0.41 | 0.42** | 0.41 | 0.42** | **0.46** | **0.36** | 0.32 | 0.34 |
| Heschl | 0.39 | 0.41** | 0.37 | 0.40** | **0.67** | **0.29** | **0.45** | **0.45** |
| Temporal_Sup | 0.40 | 0.42** | 0.38 | 0.40** | 0.28 | **0.33** | 0.13 | **0.38** |
| Temporal_Pole_Sup | 0.37 | 0.38** | 0.35 | 0.37** | **0.70** | **0.31** | **0.35** | **0.32** |
| Temporal_Mid | 0.39 | 0.41** | 0.39 | 0.41** | 0.23 | 0.28 | **0.26** | **0.46** |
| Temporal_Pole_Mid | 0.38 | 0.39** | 0.37 | 0.39** | **0.62** | **0.54** | **0.35** | **0.58** |
| Temporal_Inf | 0.39 | 0.40** | 0.39 | 0.40** | **0.43** | **0.24** | **0.44** | **0.48** |

* significant change over time ($p < 0.05$)
** significant change over time ($p < 0.05/90$)
Bold: significant broad heritability ($p < 0.05$)

**Supplemental Table 5.2** – Streamline based efficiency at measurement 1 and 2

| | Left | | Right | |
|---|---|---|---|---|
| Region[sexeffect] | M 1 | M 2 | M 1 | M 2 |
| Precentral[R] | 840 | 692** | 788 | 717** |
| Frontal_Sup[L] | 867 | 830 | 788 | 778 |
| Frontal_Sup_Orb | 301 | 355** | 334 | 398** |
| Frontal_Mid[LR] | 514 | 503 | 402 | 410 |
| Frontal_Mid_Orb[R] | 283 | 346** | 266 | 293 |
| Frontal_Inf_Oper[LR] | 506 | 482* | 467 | 417** |
| Frontal_Inf_Tri[LR] | 469 | 443* | 422 | 419* |
| Frontal_Inf_Orb[LR] | 275 | 268 | 285 | 249* |
| Rolandic_Oper[LR] | 403 | 394* | 324 | 287 |
| Supp_Motor_Area | 1088 | 962** | 1024 | 868** |
| Olfactory[LR] | 178 | 166 | 175 | 142** |
| Frontal_Sup_Medial[L] | 810 | 793 | 679 | 703 |
| Frontal_Med_Orb | 322 | 328 | 384 | 414 |
| Rectus | 183 | 168 | 194 | 165** |
| Insula[LR] | 330 | 345 | 289 | 331 |
| Cingulum_Ant | 650 | 774** | 765 | 894** |
| Cingulum_Mid | 958 | 790** | 1214 | 1022** |
| Cingulum_Post | 507 | 547* | 590 | 618 |
| Hippocampus[R] | 279 | 282 | 331 | 318 |
| ParaHippocampal[LR] | 256 | 211** | 263 | 236* |
| Amygdala[LR] | 295 | 262** | 336 | 296** |
| Calcarine | 374 | 383 | 428 | 436 |
| Cuneus[R] | 487 | 508 | 517 | 548 |
| Lingual | 289 | 285 | 297 | 286* |
| Occipital_Sup | 523 | 540 | 495 | 525 |
| Occipital_Mid[LR] | 324 | 370** | 317 | 346 |
| Occipital_Inf[R] | 242 | 256 | 278 | 263* |
| Fusiform[LR] | 290 | 236** | 348 | 294** |
| Postcentral[LR] | 548 | 446** | 659 | 590** |
| Parietal_Sup | 529 | 441** | 540 | 489** |
| Parietal_Inf[LR] | 519 | 438** | 538 | 514 |
| SupraMarginal[LR] | 640 | 612 | 573 | 527* |
| Angular[LR] | 411 | 397 | 521 | 505 |
| Precuneus[R] | 619 | 615 | 698 | 671 |
| Paracentral_Lobule[R] | 928 | 727** | 828 | 707** |
| Caudate[LR] | 348 | 287** | 380 | 309** |
| Putamen[LR] | 555 | 541 | 490 | 484* |
| Pallidum[LR] | 465 | 474 | 471 | 481 |
| Thalamus[LR] | 534 | 499* | 517 | 462** |
| Heschl | 194 | 207 | 181 | 215** |
| Temporal_Sup[LR] | 351 | 358 | 298 | 315 |
| Temporal_Pole_Sup | 287 | 297 | 260 | 286 |
| Temporal_Mid[LR] | 358 | 360 | 374 | 366* |
| Temporal_Pole_Mid[LR] | 279 | 276 | 304 | 313 |
| Temporal_Inf[LR] | 355 | 306** | 348 | 285** |

*significant change over time ($p < 0.05$)
**significant change over time ($p < 0.05/90$)
[L / R]Significant effect of sex for left (L) and/or right (R) region: boys > girls in all cases.

**Supplemental Table 5.3** – Broad heritability of streamline count based local efficiency at measurement 1 and 2, and the broad heritability of change in local efficiency.

| | Left | | Right | | Left | Right |
|---|---|---|---|---|---|---|
| Region | M 1 | M 2 | M 1 | M 2 | Change | Change |
| Precentral | 0.25 | **0.36** | **0.33** | **0.46** | 0.15 | 0.11 |
| Frontal_Sup | 0.27 | **0.35** | **0.34** | **0.31** | 0.28 | 0.11 |
| Frontal_Sup_Orb | 0.31 | **0.47** | **0.40** | **0.35** | **0.46** | **0.45** |
| Frontal_Mid | **0.56** | 0.02 | 0.24 | 0.01 | **0.24** | 0.15 |
| Frontal_Mid_Orb | 0.18 | 0.34 | 0.09 | **0.40** | 0.15 | **0.27** |
| Frontal_Inf_Oper | 0.30 | 0.24 | 0.12 | 0.00 | 0.28 | 0.13 |
| Frontal_Inf_Tri | 0.25 | 0.24 | 0.01 | 0.11 | 0.00 | 0.07 |
| Frontal_Inf_Orb | 0.07 | 0.00 | 0.18 | 0.11 | 0.04 | 0.04 |
| Rolandic_Oper | **0.34** | **0.24** | **0.44** | 0.26 | 0.02 | **0.40** |
| Supp_Motor_Area | 0.20 | 0.19 | **0.43** | **0.29** | **0.30** | 0.20 |
| Olfactory | **0.39** | **0.43** | **0.37** | 0.19 | 0.18 | 0.33 |
| Frontal_Sup_Medial | 0.21 | **0.48** | **0.31** | 0.24 | 0.22 | 0.24 |
| Frontal_Med_Orb | 0.13 | **0.39** | **0.40** | **0.64** | 0.26 | 0.19 |
| Rectus | **0.44** | **0.30** | 0.09 | 0.24 | 0.16 | 0.04 |
| Insula | 0.21 | 0.02 | 0.24 | **0.31** | 0.06 | 0.26 |
| Cingulum_Ant | **0.49** | **0.48** | **0.32** | **0.54** | **0.54** | **0.32** |
| Cingulum_Mid | **0.47** | **0.42** | **0.41** | **0.29** | **0.32** | **0.39** |
| Cingulum_Post | **0.43** | **0.34** | **0.55** | **0.37** | 0.09 | 0.22 |
| Hippocampus | **0.39** | **0.27** | **0.50** | **0.21** | 0.01 | 0.18 |
| ParaHippocampal | **0.33** | 0.08 | **0.33** | **0.10** | 0.10 | 0.09 |
| Amygdala | **0.53** | **0.29** | 0.15 | 0.00 | 0.06 | 0.09 |
| Calcarine | **0.36** | **0.37** | **0.64** | **0.36** | 0.11 | 0.18 |
| Cuneus | **0.45** | 0.16 | **0.42** | **0.22** | 0.16 | 0.02 |
| Lingual | **0.43** | **0.58** | **0.43** | 0.26 | 0.20 | 0.32 |
| Occipital_Sup | **0.46** | **0.33** | **0.39** | **0.27** | 0.14 | 0.01 |
| Occipital_Mid | **0.29** | **0.46** | **0.31** | **0.24** | 0.28 | 0.00 |
| Occipital_Inf | **0.38** | **0.24** | **0.56** | **0.36** | 0.02 | 0.31 |
| Fusiform | **0.60** | 0.13 | **0.30** | **0.25** | **0.20** | 0.02 |
| Postcentral | **0.61** | **0.18** | **0.46** | 0.15 | **0.27** | 0.28 |
| Parietal_Sup | **0.56** | **0.41** | **0.35** | 0.22 | 0.26 | 0.17 |
| Parietal_Inf | **0.51** | 0.10 | **0.45** | **0.33** | **0.54** | 0.04 |
| SupraMarginal | **0.54** | **0.39** | **0.41** | **0.24** | 0.22 | 0.06 |
| Angular | **0.24** | 0.14 | **0.31** | 0.16 | 0.02 | 0.05 |
| Precuneus | **0.41** | **0.34** | **0.39** | **0.31** | 0.01 | 0.01 |
| Paracentral_Lobule | **0.27** | **0.39** | **0.35** | 0.22 | **0.33** | **0.43** |
| Caudate | **0.45** | 0.20 | 0.22 | 0.00 | 0.26 | 0.17 |
| Putamen | **0.40** | **0.18** | **0.53** | **0.21** | 0.03 | 0.17 |
| Pallidum | **0.40** | **0.26** | **0.42** | **0.26** | 0.05 | 0.09 |
| Thalamus | **0.51** | **0.41** | 0.32 | 0.02 | 0.07 | 0.24 |
| Heschl | 0.02 | 0.07 | 0.12 | 0.24 | 0.07 | 0.12 |
| Temporal_Sup | 0.11 | **0.39** | 0.24 | 0.32 | **0.48** | 0.33 |
| Temporal_Pole_Sup | 0.17 | 0.01 | 0.34 | 0.00 | 0.09 | 0.30 |
| Temporal_Mid | 0.25 | 0.22 | 0.21 | **0.37** | 0.14 | 0.08 |
| Temporal_Pole_Mid | 0.31 | 0.06 | 0.31 | 0.06 | 0.05 | 0.08 |
| Temporal_Inf | **0.45** | 0.05 | **0.53** | **0.22** | 0.18 | 0.13 |

Bold: significant broad heritability ($p < 0.05$)

**Supplemental Table 5.4** – Significant ($p$< 0.05) phenotypic correlations (Rph) between change in streamline-based local efficiency and change in IQ.

| Region | Rph |
|---|---|
| Frontal_Inf_Orb_R | -0.21 |
| Frontal_Med_Orb_R | -0.18 |
| Rectus_R | -0.16 |
| Cingulum_Ant_L | -0.17 |
| Cingulum_Ant_R | -0.18 |
| Cingulum_Post_R | -0.15 |
| Calcarine_R | -0.18 |
| Occipital_Mid_L | -0.19 |
| Occipital_Inf_L | -0.17 |
| SupraMarginal_R | -0.16 |
| Caudate_L | -0.19 |

Chapter 6

# Association between structural brain network efficiency and intelligence increases during adolescence

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In revision

**ABSTRACT**

Adolescence represents an important period during which considerable changes in the brain take place, such as increases in integrity of white matter bundles, and increasing efficiency of the structural brain network. A more efficient structural brain network has been associated with higher intelligence. Whether development of structural network efficiency is related to intelligence, and if so to which extent genetic and environmental influences are implicated in their association, is not known.

In a longitudinal study, we mapped FA-weighted efficiency of the structural brain network in 310 twins and their older siblings at ages 10, 13, and 18 years. Age-trajectories of global and local FA-weighted efficiency were related to intelligence. Contributions of genes and environment were estimated using structural equation modeling.

Efficiency of brain networks changed in a non-linear fashion from childhood to early adulthood, increasing between 10 and 13 years, and leveling off between 13 and 18 years. Adolescents with higher intelligence had higher global and local brain network efficiency. The dependency of FA-weighted global efficiency on longitudinally stable IQ increased during adolescence ($r_{ph}$=0.003 at age 10; 0.24 at age18). Global efficiency was significantly heritable during adolescence (broad sense heritability of 43% at age 18). The genetic correlation between intelligence and global and local efficiency increased with age, with genes explaining up to 87% of the observed correlation at age 18.

In conclusion, the brain's structural network differentiates depending on IQ during adolescence, and is under increasing influence of genes that are also associated with intelligence as it develops from late childhood to adulthood.



**INTRODUCTION**

Adolescence represents a period of considerable brain development. During adolescence the cortex becomes thinner (Brown et al., 2012; Ducharme et al., 2012; Gogtay et al., 2004; Shaw et al., 2008; van Soelen et al., 2012a; Sowell, 2004) while its connecting white matter fibers increase in volume (Brouwer et al., 2012; Paus, 2010) – which it continues to do so into young adulthood (Lebel and Beaulieu, 2011; Peters et al., 2014; Schmithorst and Yuan, 2010; Simmonds et al., 2014). Together, these white matter fibers form structural brain networks with an efficient organization that becomes increasingly more efficiently organized during development (Koenis et al., 2015). Determining the process of development of the brain network during puberty is an important step in understanding developmental disorders that have their onset during this period of rapid changes (Paus et al., 2008). Because white matter network development and cognitive development go hand in hand (Koenis et al., 2015) and brain and experience may shape each other (Park and Friston, 2013; Paus, 2013), it is of importance to study both brain and cognitive development and their associations during adolescence.

Structural brain efficiency has been associated with cognitive functioning. Adults with a higher intelligence have a more efficient functional brain network (van den Heuvel et al., 2009; Langer et al., 2012) and structural white matter network (Bohlken et al., 2016b; Chiang et al., 2009; Li et al., 2009; Wen et al., 2011). Already in childhood (Kim et al., 2016) and early adolescence (Koenis et al., 2015; Schmithorst et al., 2005; Tamnes et al., 2011; Wang et al., 2012), positive associations between intelligence and structure of the white matter seem

to be present, at least to some extent. How does the brain develop during this period of major maturational changes in cognition and social environment (Blakemore et al., 2010; Luna et al., 2015)? A longitudinal setup with measurements from late childhood and throughout adolescence allows us to study this question. By including twins and their siblings, the influences of genes and environment on these developmental changes can be assessed over time.

Recently we showed in a longitudinal twin study with measurement from late childhood to early adolescence that the FA-weighted structural brain network becomes more efficient between the ages of 10 and 13, and that changes in intelligence were related to changes in structural efficiency (Koenis et al., 2015). Because we found that FA-weighted network efficiency was related to IQ whereas the streamlines-based network efficiency was not, we chose to examine the relation between IQ and FA-based network efficiency in the current study. Recently, we rescanned these twins and their siblings for a third time at age 17 (twins) and age 15-23 years (siblings) which allowed us to examine the development between FA-based network efficiency and intelligence from late childhood up to late adolescence. Here we report on developmental patterns of white matter efficiency up to early adulthood, on the association between white matter network efficiency and intelligence, and on the extent to which genetic and the environmental factors influence this development of structural brain efficiency.

## MATERIALS AND METHODS

### *Participants*

A total of 226 twins [98 monozygotic subjects (48 boys, 50 girls), 128 dizygotic subjects (66 boys, 62 girls)] and 103 of their older siblings and 1 younger sibling (44 boys, 60 girls) were included in the BrainSCALE cohort (van Soelen et al., 2012b). The sample was recruited from the Netherlands Twin Register (van Beijsterveldt et al., 2013). At the first wave the mean (SD) age was 9.9 (1.4) years; at the second wave 12.9 (1.4) years; at the third wave 17.9 (1.3) years. Interval between waves one and two was 2.93 (0.23) years, and between two and three 5.02 (0.32) years (Figure 6.1). From 20 participants, no DTI scan with sufficient quality was collected. In total, 699 scans were collected from 310 participants: 51 participants had 1 scan; 129 participants had 2 scans; 130 participants had 3 scans. Zygosity of the twins was confirmed by genome-wide SNP data. Written informed consents were obtained from all subjects and their parents. The Dutch Central Committee on Research involving Human Subjects (CCMO) approved the study.

### *Cognition*

Intelligence was assessed based on the intelligence quotient (IQ) as measured with the Wechsler Intelligence Scale for Children III (WISC-III, Dutch version) at measurement 1 and 2, and with the Wechsler Adult Intelligence Scale III (WAIS-III, Dutch version) at measurement 3. At the first measurement, all subtasks of the WISC were included. At the second measurement, six subtasks of the WISC-III were administered: four verbal subtests (similarities, arithmetic, vocabulary, and digit span), and two non-verbal subtests (picture completion and block design). At the third measurement, four subtasks of the WAIS-III were administered: similarities, vocabulary, block design, and matrix reasoning. Scores on subtests from the WISC or WAIS were standardized against age-specific norms, leading to a total IQ

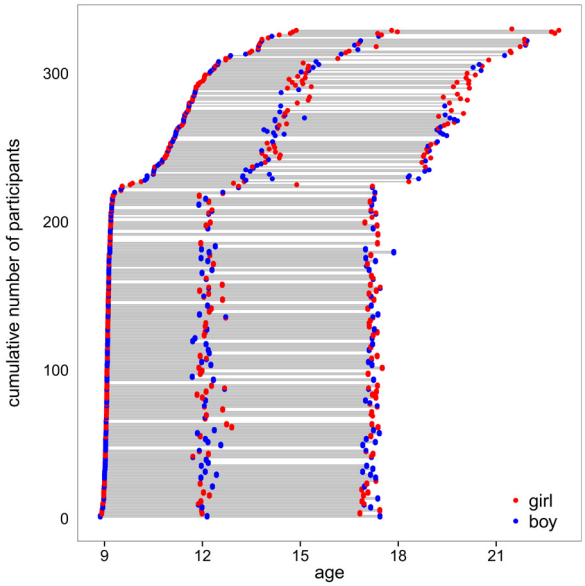


**Figure 6.1** – Ages of the individual participants from the twin families and their older singleton sibling at the three measurements. Individuals that participated multiple times are connected by lines.

score. Because we were interested in the stable IQ factor during adolescence, a mean IQ over all measurements was computed for each individual, including all available IQ scores for that participant. Based on their mean IQ, participants were divided in three equally sized IQ groups: below average (mean IQ <= 96, n=111, group mean (sd): 86.9 (7.0)), average (mean IQ 97-106, n=111, group mean= 101.5 (3.0)), and above average (mean IQ >106, n= 108, group mean= 116.2 (8.7)). On a group level, mean IQ was 101.4 (13.7). Per measurement, mean (sd) of IQ was: 101.9 (14.7); 101.7 (15.3); 103.8 (12.7).

***MRI acquisition***

All MRI brain scans were acquired at the University Medical Center Utrecht on a 1.5 Tesla Philips Achieva scanner (Philips, Best, The Netherlands) using the same protocol at all waves (Brouwer et al., 2012). For anatomy, a three-dimensional T1-weighted scan (Spoiled Gradient Echo; TE = 4.6 ms; TR = 30 ms; flip angle 30°; 160–180 contiguous coronal slices of 1.2 mm; in-plane resolution 1 x 1 $mm^2$; acquisition matrix 256 x 256) of the whole head was made of each individual. For white matter fiber tracking two Single Shot Echo Planar Imaging (SS-EPI) DWI scans were acquired (32 diffusion-weighted volumes with diffusion weighting b = 1000 s/$mm^2$ and 32 non-collinear diffusion gradient directions; 8 diffusion-unweighted (b = 0 s/$mm^2$) scans; TE = 88 ms; TR = 9822 ms; parallel imaging SENSE factor 2.5; flip angle 90°; 60 transverse slices of 2.5 mm, no gap, FOV 240 mm; 128 x 128 reconstruction matrix; 96 x 96 acquisition matrix, no cardiac gating) for optimal signal-to-noise ratio.

***MRI processing***

White matter pathways, referred to as fibers or tracts, were reconstructed using streamline tractography. First, the 2 DWI scans were combined and corrected for possible gradient-induced distortions (Andersson and Skare, 2002). Next, the diffusion pattern in each voxel was fitted to a tensor matrix using a robust M-estimator (Chang et al., 2005), providing three eigenvectors (representing the three principal directions of diffusion) and corresponding eigenvalues. Fractional anisotropy (FA) values were calculated in each voxel as a measure of microstructural directionality from the eigenvalues (Basser and Pierpaoli, 1996). Then, the b0 scan was registered to the T1-weighted scan using a rigid transformation (no scal-

ing), based on optimization of a mutual information metric (Maes et al., 1997), and the T1-weighted images were nonlinearly warped into model space per measurement up to a scale of 1 mm (Collins et al., 1995), and then to the model of the second measurement. All possible fiber tracts between two regions were reconstructed in individual space using the diffusion tensor images with an in-house implementation of the fiber assignment by a continuous tracking (FACT) algorithm (Mori and Van Zijl, 2002) with 8 seed points per voxel, FA threshold of 0.1 and maximal angle of 45 degrees. The fiber tracts were warped into model space, using the concatenation of the transformations between the b0 scan and T1 scan, and between the T1 scan and model space. For network construction, the AAL template (Tzourio-Mazoyer et al., 2002) was warped onto the model brain, segmenting the cortex in a parcellation map consisting of 90 regions.

***Construction of structural brain networks***

A network consists of a set of nodes and connections (edges) that can be mathematically expressed as a graph with a collection of nodes and a collection of edges between the nodes (Bullmore and Bassett, 2011). Whole brain networks were created based on the 90 AAL brain regions. Structural FA-weighted brain networks were created for each individual, one for each measurement when available. Each individual network included bundles that were present in at least 60% of all participants in each wave (de Reus and van den Heuvel, 2013): Network nodes *i* and *j* were defined as structurally connected by an edge when from the total collection of reconstructed streamlines, in at least 60% of the participants of wave 1, wave 2, and wave 3 at least one fiber connected region *i* and *j*. For each edge, each subject and each wave, weight $w_{ij}$ was defined by the mean FA value of the traced fibers between region *i* and *j*.

***Graph analysis***

Mathematical representations of the structural connectivity of the human brain network have revealed that the brain is organized according to a highly efficient small-world topology combining a high level of segregation (local efficiency) with a high level of global integration (global efficiency) (Achard and Bullmore, 2007). Global efficiency is a network attribute that quantifies how easy information can be exchanged over the network, providing information on the communication efficiency of a network as a whole. Local efficiency reflects how well information can travel in the direct neighborhood of a node, and is often interpreted as a metric of the local information processing capacity of a network (Bullmore and Sporns, 2012; van den Heuvel and Hulshoff Pol, 2010). To measure changes in efficiency, we computed global and local efficiency in the AAL regions for each individual at each measurement using the Brain Connectivity Toolbox (Rubinov and Sporns, 2010; http://www.brain-connectivity-toolbox.net). Figures were created with the BrainNet Viewer (Xia et al., 2013).

***Statistical analyses***

Structural equation modeling, implemented in the OpenMx package (Boker et al., 2011) in R (R Core Team, 2014), was used in all analyses to account for dependency of the data and to estimate the relative influences of genes and environment. In a first step, the best fitting age-trajectory of global and local efficiency was determined using a step-wise approach:

we entered efficiency data up to three measurements for each subject in a saturated model, allowing for non-zero covariances between measurements and between members of twin and twin-sib pairs. We subsequently fitted a cubic, quadratic, linear and constant age-model, with age entered as continuous measure, for each network measure in a top-down fashion.

If the quadratic model fitted worse than the cubic model (based on the differences between the -2 log likelihoods, $p < 0.05$), the best model was assumed to be cubic, if not, this quadratic model was then compared to a linear model, and so on. Once the best-fitting model for each network measure was determined, we proceeded in two ways:

1) Based on the best-fitting age model (cubic, quadratic, linear, or constant), we tested whether a model allowing different trajectories for the three IQ groups fitted the data better than the model using only one trajectory for the whole group.

2) We computed residuals of the network measures by taking out the effect of age based on the best fitting model determined above. The residuals were subsequently modeled in a 4-variate genetic model (described below), combining the network data per wave and mean IQ, to estimate a) (genetic) correlations between network measures and IQ at each of the three waves; b) heritability of network measures at the three waves; c) the genetic correlations between network measures at wave 1 and 2, and between wave 2 and 3.

***Genetic modeling of twin and sib data***

Relative influences of genetic and environmental factors were examined in an extended twin design by comparing within-pair correlations between monozygotic (MZ) and dizygotic (DZ) twins / twin sibling pairs. Differences between these correlations may arise because monozygotic twins share (almost) 100% of their genetic makeup and twin-sibling pairs, like dizygotic pairs, share on average 50% of their segregating genes (Posthuma et al., 2003). When an MZ correlation is twice as high as a DZ correlation, this indicates that familial resemblance is largely accounted for by genetic factors. In addition to genetic factors, resemblance between twins and sibs can arise from common environmental factors, which comprises those environmental factors that induce similarity in children growing up in the same family. The presence of common environmental factors is suggested when correlations between DZ twins and twins/sibs are larger than half the MZ correlation. When the MZ correlations are more than twice the DZ/sib correlations, there is a suggestion for non-additive genetic influences (for example epistasis or dominance). Unique environmental influences are not shared with other family members and also contain the measurement error.

The same rationale as described for the univariate case can be applied to multivariate data. If a correlation exists between two variables, the cross-trait cross-twin/sibling correlations give an indication whether the same genes, or (shared) environment is responsible for the association. Estimates of heritability of the network and correlations with IQ were done in a 4-variate model: network metric of each measurement (thus three), and mean IQ.

***Genetic analyses***

For each network measure, we fitted a 4-variate model to the data including the three network measures at each wave and mean IQ. Based on our previous work, we fitted a model that allowed for additive and non-additive genetic effects and unique environment for the network measures (Koenis et al., 2015) and additive genetic effects, and common and

unique environment for mean IQ (van Soelen et al., 2011) (Supplementary Figure 6.1). The proportion of the total variance that can be attributed to genetic or environmental factors gives estimates of (univariate) heritability ($h^2$), broad sense heritability ($h^2 + d^2$), common environmental influence ($c^2$) and unique environmental influence ($e^2$). A genetic correlation $r_g$ between each pair of two variables was defined as the (broad) genetic covariance between two traits, divided by the square roots of the part of variances that can be attributed to (broad) genetic factors for each trait (for details see Koenis et al., 2013). Because a genetic correlation does not take the heritability of the traits into account, we also computed the $r_{ph}$-g which is defined as the (broad) genetic covariance between two traits, divided by the square roots of the variances for each trait (Toulopoulou et al., 2007). $R_{ph}$-g can be interpreted as the correlation that would be observed if only genetic factors are taken into account. The environmental correlations $r_e$ and $r_{ph}$-e were defined similarly.

***Post-hoc analyses***

In our previous work, we found that changes in IQ are related to changes in FA-based local efficiency between the age of 10 and 13 years (Koenis et al., 2015). Here, we focused on the stable component of IQ, by averaging all available IQ measures of a subject. However, in a post-hoc analysis, we investigated whether change in IQ and change in local efficiency were related between the ages of 13 and 18 years. Additionally, we recomputed the correlations with IQ using the IQ measure acquired at each wave.

As another post-hoc analysis, we tested whether the developmental pattern of the structural brain network was different for boys and girls. Subsequently, we tested whether the correlations between efficiency measures and IQ were different in boys and girls, by testing a genetic model that allows for qualitative sex differences. In such a model, the covariance between members of opposite-sex twin and twin-sibling pairs that is attributed to additive (or dominant) genetic factors is not assumed to be equal to 0.5 (0.25 for dominant factors), but estimated from the data. Sex differences in additive and dominant genetic influences on the covariance were tested in separate models. Quantitative sex effects were tested in a model in which the genetic factors influencing network measures in boys and girls were the same, but the path loadings (i.e. heritabilities) were allowed to be different.



**RESULTS**

***Efficiency of adolescent brain networks develops in a non-linear pattern***

FA-based global efficiency followed a cubic pattern (better fit compared to lower degree polynomial functions; $p = 0.0003$) with age, characterized by an increase in efficiency from age 10 to around age 13, followed by a period of leveling off until around age 18 (Figure 6.2). On a local level, most regions showed this cubic pattern, although for some regions a quadratic trajectory fitted the data better (Supplementary Figure 6.2). Visually, regional differences in developmental trajectories were mostly observed between the ages of 13 and 18, where FA-based local efficiency either decreased or remained stable: in frontal areas the decrease was most prominent, in subcortical areas milder decrease in efficiency was found, while decrease was minimal or absent in temporal, parietal and occipital areas (Supplementary Figure 6.2).

***Adolescent brains differentiate in relation to IQ***

When separating out age trajectories per IQ group, differential trajectories of global efficiency for the three IQ groups revealed a better fit ($p = 0.04$) than one trajectory for all participants. More specifically, we found that the high IQ group was best characterized by stable global efficiency between the ages 13 through 18, whereas the middle and lower IQ groups on average decline in global efficiency (Figure 6.3A). A differential pattern for each IQ group was seen also for local efficiency in numerous regions throughout the brain (54 regions; FDR-corrected for 90 brain regions, $p < 0.05$) (Figure 6.3B).

***Correlations between IQ and efficiency emerge during adolescence***

Over time, the association between IQ and global and local FA-weighted network efficiency increased significantly (Figure 6.4): A model which allowed free estimations of phenotypic correlations between efficiency and IQ fitted better than a model which constrained the correlations to be equal across measurements (global efficiency $p=0.02$; local efficiency in 46 regions, $p < 0.05$; 12 regions reached FDR corrected significance). More specifically, at age 10, efficiency of the structural network was not significantly correlated with IQ (global efficiency: $r_{ph} = 0.003$; $p = 0.96$). At age 13, IQ was significantly correlated with global efficiency ($r_{ph} = 0.15$, $p = 0.05$) and this correlation was even higher at age 18 ($r_{ph} = 0.24$, $p = 0.002$) (Figure 6.4B). At the local level, there was one region with a significant association between local efficiency and IQ at age 9 ($rph = 0.13$, $p = 0.05$). At age 13, local efficiency was associated with IQ in 35 regions of the brain (mean local efficiency $rph = 0.16$, $p = 0.03$; regionally in 36 regions up to $rph = 0.21$, $p=0.006$) but these correlations did not survive FDR-corrected significance. At age 18, correlations between local efficiency and IQ at the regional level 74 regions reached FDR-corrected significance (mean local efficiency: $r_{ph} = 0.26$, $p = 0.0007$, regionally in 74 regions, up to $r = 0.29$, $p = 0.0002$; see Supplemental Table 6.1).

Across measurements, the correlations between global efficiency and IQ were partly driven by genes (Figure 6.4B). The relative influence of genes implicated in the association between intelligence and FA-weighted global efficiency increased significantly over time: a model which constrained the genetic part of the phenotypic correlation ($r_{ph}$-g) to be equal across all measurements fitted worse than a model which allowed the correlation to be fitted freely (global efficiency: $p = 0.02$). Investigating the three waves separately, $r_{ph}$-g between FA-weighted global efficiency and IQ was not significantly different from zero at the first wave ($r_{ph}$-g = 0.026, $p = 0.76$), but it was at waves 2 and 3 (wave 2: $r_{ph}$-g = 0.25, $p = 0.004$; wave 3: $r_{ph}$-g = 0.20, $p = 0.016$), to an equal extent ($r_{ph}$-g could be set equal at wave 2 and 3, $p = 0.63$). In addition, in wave 2, around the age of 13, but not in wave 3 (around age 18) there was a unique environmental influence that annulled the genetic association between FA-weighted global efficiency and IQ ($r_{ph}$-e = -0.10; $p = 0.02$).

Positive contributions of genes to the correlations between IQ and FA-weighted local efficiency were found at ages 13 and 18 (age 13: 71 regions, $p < 0.05$ FDR corrected; age 18: 60 regions at $p < 0.05$, of which 18 regions reached FDR corrected significance, Supplementary Figure 6.3). The same pattern of an increase in the genetic contribution to the correlation between local efficiency and IQ from age 10 to age 13 (32 regions $p < 0.05$ uncorrected) and then a stable positive $r_{ph}$-g to age 18 (all regions, $p > 0.08$) was seen throughout the brain. Similar to the correlation between IQ and global efficiency, locally, a negative

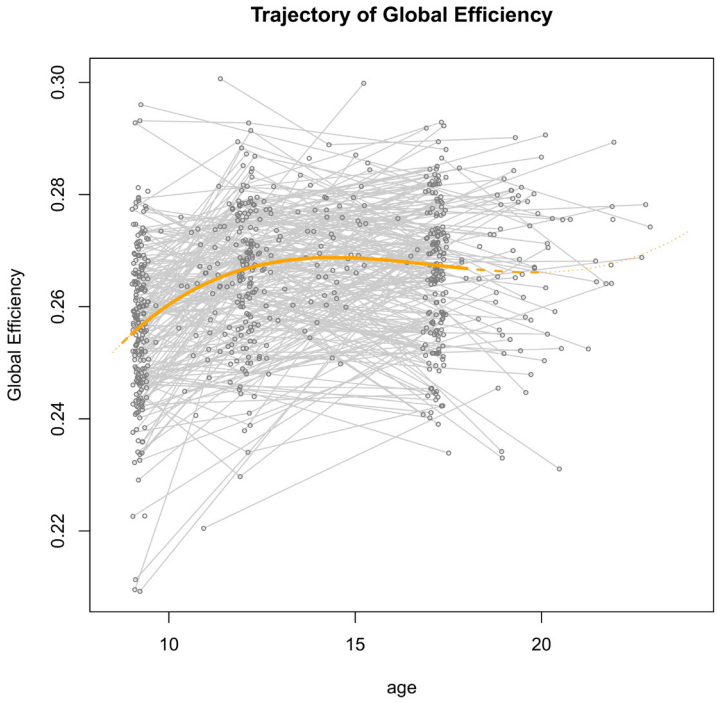


**Figure 6.2** – Development of FA-weighted global efficiency during



unique-environmental correlation between IQ and FA-weighted local efficiency was found at age 13, throughout the brain in 43 regions ($r_{ph}$-e up to –0.14, p = 0.0005 uncorrected, see Supplemental Figure 6.3).

***Genetic influences on structural network efficiency and IQ***

Broad heritability of FA-weighted global efficiency was 39% (95% CI: 14-59%), 20[5-43]%, and 43[19-62]% for ages 10, 13 and 18 respectively (Figure 6.5A). Broad heritability did not significantly differ at the respective ages for global and local efficiency (Figure 6.5B; Supplemental Table 6.2). Heritability of mean IQ was estimated at 72% (95% CI: 54-92%).

A stable genetic factor influenced global efficiency at waves 1 and 2 ($r_g$=0.78 [0.09-0.98]). A model where $r_g$ was constrained to be zero fitted worse than a model that allowed $r_g$ to be estimated freely (p=0.03). The same was found for the genetic correlation between global efficiency at wave 2 and 3 ($r_g$=0.85 [0.25-1.00], p = 0.008).

For local efficiency, 38 regions were partly influenced by the same genetic factor at both wave 1 and 2 (p < 0.05, FDR-corrected, see Supplemental Table 6.2). For waves 2 and 3, 51 regions were found to be influenced by the same genetic factor (p < 0.05, FDR-corrected). Regions where a stable genetic factor influenced local efficiency were distributed over the entire brain.

***Post-hoc analyses***

Repeating the analyses by computing the associations between IQ and brain network measures

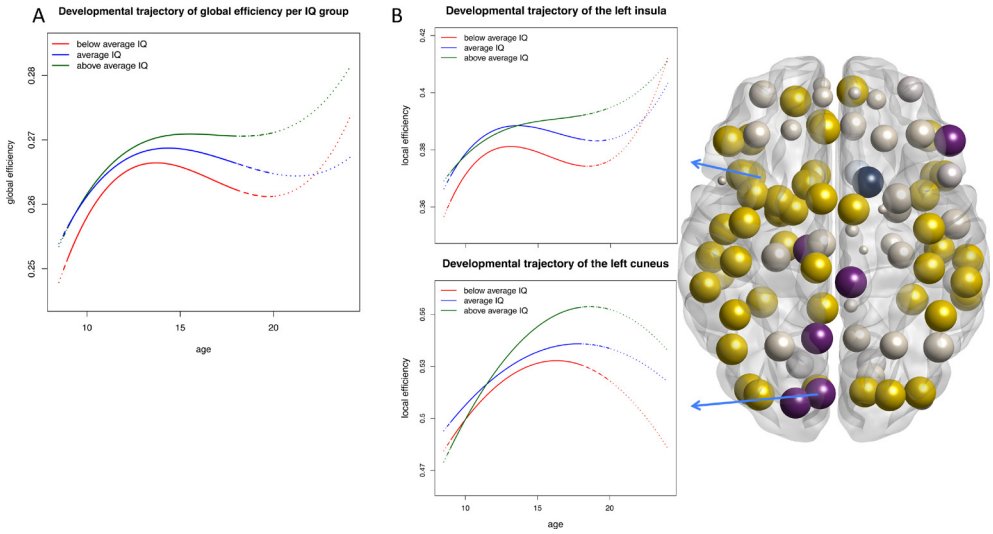


**Figure 6.3** – Development of efficiency depends in part on intelligence and genes. Global (A) and local (B) FA-weighted efficiency develops differentially depending on IQ. A: Trajectories of global efficiency for the three IQ groups. B: Regions were local efficiency of different IQ groups followed significant (FDR corrected; in yellow, purple, and blue) differential trajectories. Sizes of the spheres reflect relative p-value (1 – FDR-corrected p value). Yellow spheres indicate cubic fit; purple quadratic fit; blue a linear fit. Insets show an example of the local efficiency of the left cuneus and left insula.



using the IQ measured at that wave instead of the mean IQ, did not change our findings.

There were no significant differences between boys and girls at each time-point separately for FA-weighted global and local efficiency. The associations between global and local efficiency of the brain with IQ revealed no evidence for quantitative or qualitative genetic sex-differences.

No differential developmental trajectories for FA-weighted global efficiency in boys and girls were found (p= 0.11). Locally, however, several regions showed a better fit for the model with different trajectories for boys and girls compared to the model that fitted one trajectory for all participants. This sex-effect reached FDR-corrected significance ($p < 0.05$) for the following 10 regions: left rolandic operculum, left medial orbitofrontal gyrus, left anterior cingulum, left amygdala, bilateral calcarine sulcus, right angular gyrus, right pallidum, right heschl gyrus, left superior temporal gyrus. Generally, in these 10 regions, boys started with a lower efficiency than girls at age 10, made a steeper increase in efficiency between ages 10 and 13 and end with a higher efficiency than girls at age 13 and 18. For the left superior temporal gyrus, left rolandic operculum and right heschl gyrus, the fitted age trajectory for boys had a lower level of efficiency over the entire age range compared to girls.

The strong correlations between local efficiency and IQ at age 18 seem to be driven by the girls in our sample: 2 regions reached FDR significance in the boys, whereas 46 regions reached FDR significance in girls of the same age.

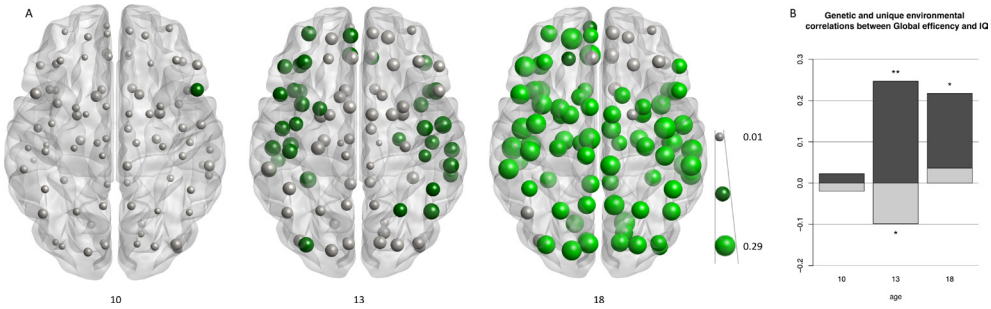


**Figure 6.4** – A: Phenotypic correlation between IQ and local efficiency at age 10, 13, and 18. Size of the spheres reflects size of the correlation. Dark green nodes indicate significance at $p < 0.05$; light green spheres indicate FDR-corrected significance. B: Genetic and unique environmental contributions to the phenotypic correlation between global efficiency and IQ at age 10, 13, and 18. * significant at $p < 0.05$; ** significant at $p < 0.005$.

## DISCUSSION

In this longitudinal study, we measured the development of FA-weighted global and local efficiency of the brain network in relation to intelligence in (young) adolescent twins and their siblings from over 100 families at 3 time-points. We show that FA-weighted efficiency of the structural brain network increases during early adolescence and levels off during mid adolescence. Moreover, during adolescence, individual differences in intelligence become increasingly reflected in the structural brain network, with widespread correlations between intelligence and FA-weighted local efficiency at age 18 years. This effect is due to development of network efficiency. Finally, we report that genes contribute to the brain's network efficiency during development and to its growing association with intelligence, explaining up to 87% of this association by age 18, while unique environmental factors counteract this genetic effect around age 13 years. Thus, during adolescent development, communication in the brain network becomes a reflection of intelligence by the age of 18 years, in part under the influence of genes.

We report for the first time that efficiency of the structural brain network increases throughout adolescent development in a non-linear fashion: an increase in efficiency that is particularly prominent before age 13, and levels off during mid-adolescence. Moreover, development of local efficiency follows different trajectories, with frontal and subcortical regions having a seemingly small decrease in local efficiency around mid-adolescence, whereas occipital regions continued to increase in efficiency until adulthood. Earlier, we showed that between 10 and 13 years there is an increase in FA-based efficiency of the brain network with most regions showing increases in efficiency over time (Koenis et al., 2015). Here we extend this finding with a third measurement in these twins and their siblings on the verge of adulthood, revealing that the increase in global and local FA-based efficiency stabilizes in late adolescence in most individuals, and decreases in some individuals. Regarding the influence of genes on network efficiency, we find that during adolescence variance in network efficiency could partly be explained by stable genetic influences.

Our finding that FA-weighted efficiency increase throughout adolescence but levels off

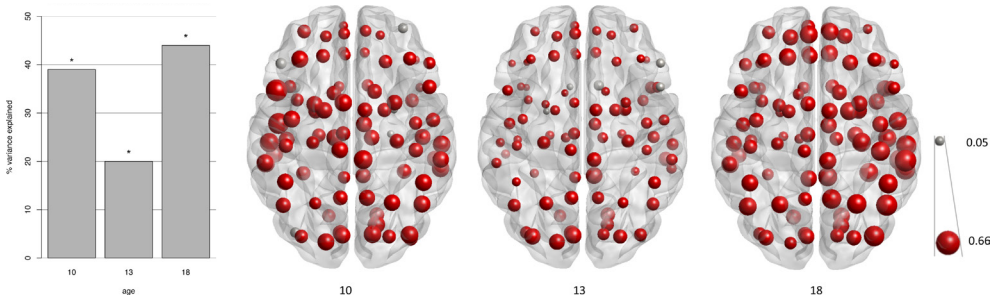


**Figure 6.5** – Broad heritability of global (A) and local (B) efficiency during adolescence. Broad heritability of global efficiency was significantly different from zero (*) on all three measurements. Size of the spheres reflects magnitude of broad heritability. Grey spheres indicate estimates of broad heritability were not significantly different from zero.

between age 13 and 18, is supported by cross-sectional studies reporting increases in FA with increasing age during adolescence and a pattern of FA development that differs across white matter bundles (Lebel and Beaulieu, 2011; Peters et al., 2014; Schmithorst and Yuan, 2010; Simmonds et al., 2014). A recent longitudinal study in adolescents between 15 and 19 years, comparable with our second and third wave, also reports both increases and decreases in FA and streamline count during late adolescence, with preferential maturation in hub-to-hub connections (Baker et al., 2015). Our findings are also in alignment with development of white matter volume (Brouwer et al., 2012; Paus, 2010) , which is consistent with the small but positive association between white matter volume and FA reported in adult twins (Bohlken et al., 2016a). Also, our FA-weighted findings align with cross-sectional reports of streamline-count weighted efficiency of brain networks showing largely positive correlations with age in childhood

Our finding that FA-weighted efficiency increase throughout adolescence but levels off between age 13 and 18, is supported by cross-sectional studies reporting increases in FA with increasing age during adolescence and a pattern of FA development that differs across white matter bundles (Lebel and Beaulieu, 2011; Peters et al., 2014; Schmithorst and Yuan, 2010; Simmonds et al., 2014). A recent longitudinal study in adolescents between 15 and 19 years, comparable with our second and third wave, also reports both increases and decreases in FA and streamline count during late adolescence, with preferential maturation in hub-to-hub connections (Baker et al., 2015). Our findings are also in alignment with development of white matter volume (Brouwer et al., 2012; Paus, 2010) , which is consistent with the small but positive association between white matter volume and FA reported in adult twins (Bohlken et al., 2016a). Also, our FA-weighted findings align with cross-sectional reports of streamline-count weighted efficiency of brain networks showing largely positive correlations with age in childhood and adolescence (Dennis et al., 2013; Hagmann et al., 2010) while leveling off or showing a negative correlation in adulthood (Dennis et al., 2013; Gong et al., 2009; Lim et al., 2015). We add to the existing literature that the adolescent FA-weighted network develops in a non-linear pattern

The question remains how the brain continues to function in an efficient manner

during the years in which both brain and cognition are changing rapidly (Blakemore et al., 2010; Luna et al., 2015). Based on our data, it seems as if the moment the structural brain network has grown into its adult state, we can see the individual levels of general cognitive functioning reflected in the brain's level of network efficiency, while earlier in development there are only some indications of this link. This is probably related to the stable component of IQ, as our findings were similar using mean IQ of all time points, or IQ at the individual time points. That developmental trajectories of white matter connectivity depend on cognitive functioning is supported by the study of Tamnes et al. (2010), which showed that the development of FA between 8-30 years is related to the level of verbal intelligence. Other studies support the notion that structural brain associations with IQ are not stable throughout life. Cortical thickness studies show differential developmental trajectories depending on the IQ (Brans et al., 2010; Brouwer et al., 2014; Karama et al., 2014; Schnack et al., 2015; Shaw et al., 2006). The absence of a correlation between FA-weighted network efficiency and IQ in late childhood is somewhat inconsistent with a recent study in in children age 6-11 years that reports a positive correlation between block design and FA-weighted network efficiency, but not with other non-verbal subtasks (Kim et al., 2016). However, total IQ versus a single IQ-subtask may be differently related to local brain regions and thus may show a different developmental pattern in relation to brain development.

Global efficiency of the white matter network is already heritable at age 10 years (39%), and remains largely stable throughout adolescence, with estimated heritability of 20% at age 13 years and 44% at age 18 years. This estimate is comparable with the heritability estimates of FA in adult twins (Blokland et al., 2012; Bohlken et al., 2014; Chiang et al., 2011; Jahanshad et al., 2013; Kochunov et al., 2015). While significant, its heritability is quite modest when compared with the heritability for white matter volume that is estimated to be over 85% in both adolescence (van Soelen et al., 2013) and adulthood (Bohlken et al., 2014). This leaves ample room for influences of the environment on the network of white matter fiber connectivity in adolescence and adulthood.

Individual differences in genetic makeup become increasingly implicated in the association between IQ and FA-weighted efficiency of the structural network: from no significant influence at age 10 years ($r_{ph}$-g = 0.02) to a significant influence at age 13 (wave 2: $r_{ph}$-g = 0.25) and remaining largely stable at age 18 (wave 3: $r_{ph}$-g = 0.20). By the time adolescents have reached age 18 years, genes explain up to 87% of the association between intelligence and local efficiency of the brain network. Individual environmental factors were found to counteract this development around age 13 years ($r_{ph}$-e = -0.10), suggesting that at that time unique environmental differences are important for this association. As IQ was assumed to be equal at all three waves, these genetic and environmental influences can be considered to be largely due to development of the brain network.

Individual increases and decreases in IQ related to brain network changes were only found in early adolescence in our cohort (Koenis et al., 2015). Changes in individual IQ over time have been reported earlier (Burgaleta et al., 2014; Ramsden et al., 2011; Waber et al., 2012). These individual changes in IQ were found to be associated with changes in brain structure (Ramsden et al., 2011) and with changes in efficiency of the brain network in early adolescence (Koenis et al., 2015). In our cohort, between ages 13 and 18, we did not find that changes in IQ were significantly related to changes in FA-weighted network efficiency.

This could be caused by the more pronounced influences of the genetic background that influences the brain and intelligence during late adolescence. For both IQ (Haworth et al., 2010; van Soelen et al., 2011) and brain structure (Lenroot et al., 2009) genetic influences have been shown to increase with age. Moreover, individuals with a higher IQ show a higher common environmental influence on IQ during adolescence compared to teenagers with a lower IQ (Brant et al., 2013). This may allow for a longer sensitive period in individuals with a higher IQ.

Girls enter puberty earlier than boys (Koenis et al., 2013; Mul et al., 2001), and their brains also develop at a different pace during adolescence (Gogtay et al., 2004; Gur and Gur, 2016; Raznahan et al., 2010). We find sex related differences in the developmental trajectories of network efficiency in a few regions, with girls starting to increase in efficiency earlier than boys and boys catching up with the girls around age 13. Indeed, several studies report differences between the sexes with regard to development of FA, especially between childhood and early adulthood (Herting et al., 2012; Ladouceur et al., 2012; Lebel et al., 2012; Schmithorst et al., 2008; Simmonds et al., 2014; Wang et al., 2012; but see Lebel and Beaulieu, 2011; Peters et al., 2014). Despite the difference in the trajectories, no significant differences between the sexes were found within each wave for mean efficiency values in our cohort. In addition, no qualitative or quantitative sex differences were found on the (genetic) association between FA-weighted local network efficiency and IQ at each wave. However, the brain wide correlation between local efficiency and IQ at age 18 seems to be more prominent in the girls in our cohort. Sex dependent associations in white matter with IQ have been reported earlier, both in children (Luders et al., 2011; Schmithorst, 2009; Wang et al., 2012) and in adults (Gur et al., 1999; Tang et al., 2010, but see Tamnes et al., 2010). Possibly, because girls start their pubertal development earlier than boys do (with regard to both secondary sexual characteristics and brain development) the difference between boys and girls at age 18 we observed, is the result of the timing of developmental processes. A fourth measurement would be able to elucidate this question.

Some limitations need to be taken into consideration when interpreting the findings of this study. First, the network measures are strongly driven by their weights, in our case FA. Although we did not correct for individual differences in mean FA of the total white matter, the results give an indication of the connectivity of the white matter bundles as well as their strength (measured by FA) of the connections. Especially for local efficiency, a network approach is more informative than measures of local FA. Second, we specifically studied the FA-weighted networks because our previous study showed a correlation between FA-weighted network efficiency and IQ, but not streamline weighed network efficiency (Koenis et al., 2015). Besides, we showed that during adolescence, the streamline weighted network behaves different from the FA-weighted network (Koenis et al., 2015). This must be taken into account when comparing the results with other studies.

In conclusion, we find non-linear development of the structural brain network's efficiency during adolescence that is differentiates with intelligence. The correlation between IQ and local FA-weighted efficiency becomes clearly visible by the time adulthood is reached, and is influenced by genes common to both intelligence and structural network efficiency.

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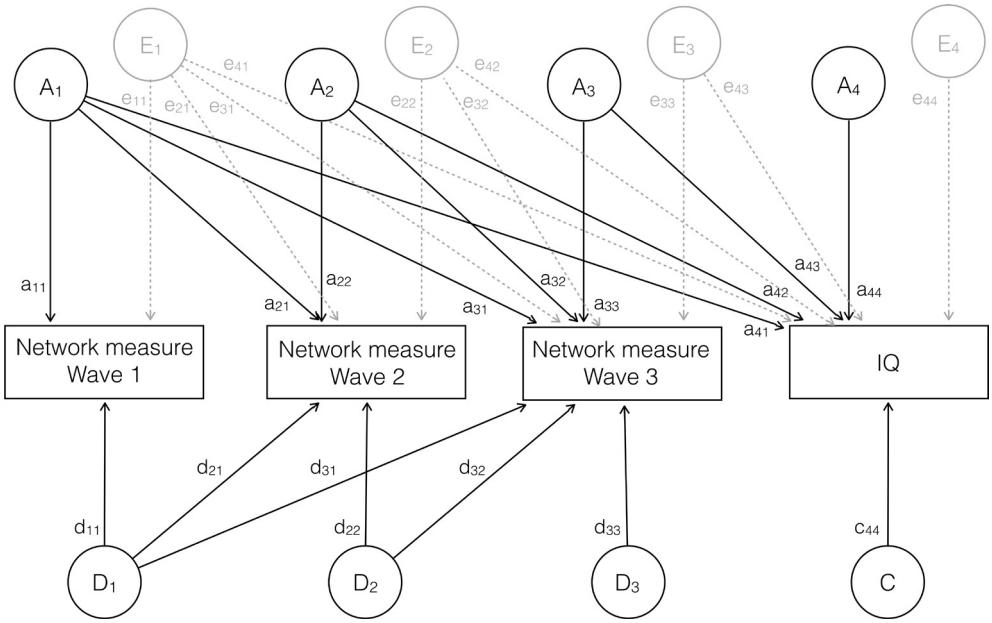
**SUPPLEMENTAL MATERIAL**

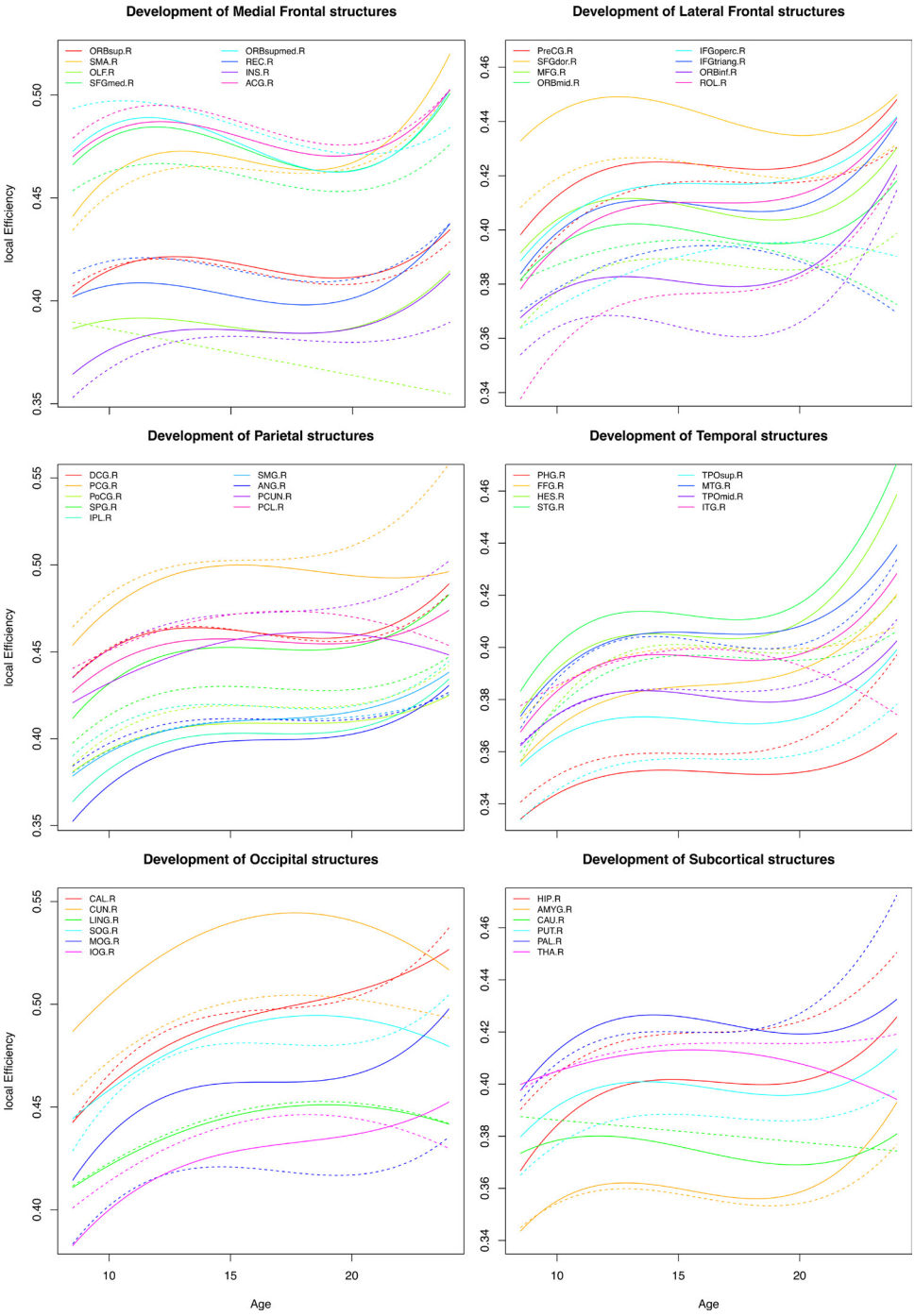



**Supplemental Figure 6.1** – Four-variate twin model to which the data was fitted. For clarity, half of the model is shown, i.e., only for twin A.

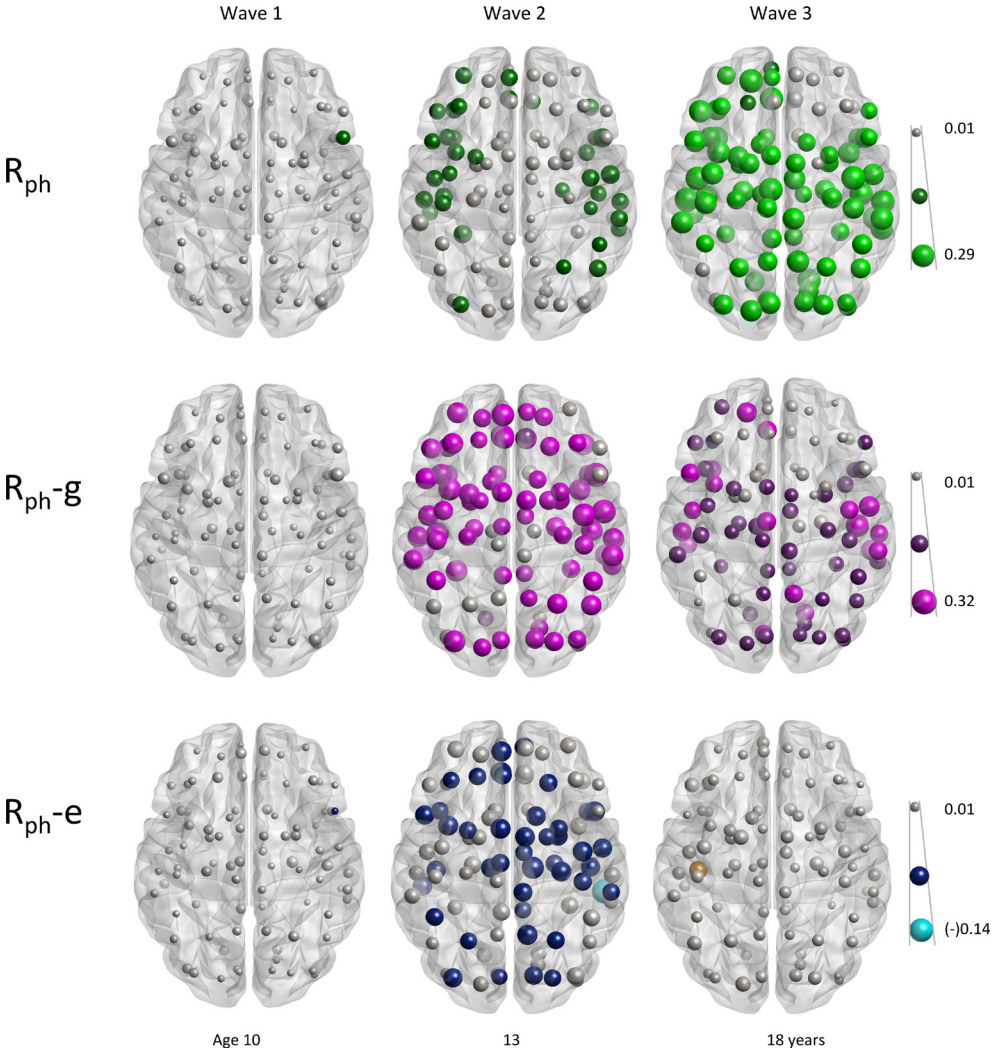
**Supplemental Table 6.1** – Phenotypic correlations between mean IQ and local efficiency at all three measurements (M1, M2, M3).

| | Left | | | Right | | |
|---|---|---|---|---|---|---|
| | Rph M1 | Rph M2 | Rph M3 | Rph M1 | Rph M2 | Rph M3 |
| Precentral | 0.02 | 0.15** | **0.25*** | 0.04 | 0.18** | **0.28**** |
| Frontal_Sup | 0.00 | 0.10** | 0.16 | -0.00 | 0.13** | 0.14 |
| Frontal_Sup_Orb | -0.02 | 0.13** | **0.28**** | -0.03 | 0.15** | 0.12 |
| Frontal_Mid | 0.02 | 0.17** | **0.19** | -0.04 | 0.12** | 0.14 |
| Frontal_Mid_Orb | 0.00 | 0.17** | **0.20** | -0.02 | 0.07 | 0.07 |
| Frontal_Inf_Oper | 0.09 | 0.16** | **0.25**** | 0.13 | 0.13 | **0.18** |
| Frontal_Inf_Tri | 0.04 | 0.17** | **0.26*** | 0.07 | 0.11 | **0.17*** |
| Frontal_Inf_Orb | 0.04 | 0.18** | **0.23*** | 0.05 | 0.18** | 0.13 |
| Rolandic_Oper | 0.04 | 0.17** | **0.27**** | 0.06 | 0.20** | **0.24**** |
| Supp_Motor_Area | -0.02 | 0.13** | **0.21*** | 0.01 | 0.13** | **0.21*** |
| Olfactory | 0.04 | 0.13** | **0.18** | 0.05 | 0.11 | 0.15 |
| Frontal_Sup_Medial | -0.01 | 0.18** | **0.17** | -0.02 | 0.12** | 0.15 |
| Frontal_Med_Orb | -0.01 | 0.08 | 0.15 | -0.01 | 0.06 | 0.12 |
| Rectus | 0.07 | 0.18** | **0.27**** | 0.02 | 0.16** | 0.13 |
| Insula | 0.06 | 0.18** | **0.26**** | 0.04 | 0.17** | **0.24*** |
| Cingulum_Ant | 0.04 | 0.14** | 0.15 | -0.00 | 0.10* | 0.15 |
| Cingulum_Mid | 0.02 | 0.10** | **0.27**** | 0.03 | 0.10** | **0.24*** |
| Cingulum_Post | 0.01 | 0.12** | **0.22*** | 0.01 | 0.10** | **0.20*** |
| Hippocampus | -0.01 | 0.15** | **0.21*** | -0.02 | 0.10** | **0.23*** |
| ParaHippocampal | 0.06 | 0.13** | **0.24*** | 0.06 | 0.15** | **0.23** |
| Amygdala | 0.06 | 0.14** | **0.18** | 0.09 | 0.15** | **0.22*** |
| Calcarine | 0.02 | 0.06 | **0.22**** | -0.01 | 0.13** | **0.22*** |
| Cuneus | 0.04 | 0.12** | **0.23*** | 0.01 | 0.13** | **0.22*** |
| Lingual | 0.03 | 0.10* | **0.22*** | 0.03 | 0.06 | **0.27**** |
| Occipital_Sup | 0.04 | 0.13** | **0.25*** | -0.03 | 0.14** | **0.22*** |
| Occipital_Mid | 0.01 | 0.17** | **0.22*** | -0.09 | 0.12** | **0.19*** |
| Occipital_Inf | -0.00 | 0.12 | 0.15 | -0.07 | 0.11 | **0.19*** |
| Fusiform | 0.03 | 0.20** | **0.20*** | -0.02 | 0.13** | **0.20*** |
| Postcentral | 0.03 | 0.17** | **0.25*** | 0.03 | 0.13** | **0.24*** |
| Parietal_Sup | 0.02 | 0.09 | **0.21** | -0.00 | 0.15** | **0.21*** |
| Parietal_Inf | -0.03 | 0.14** | **0.19** | -0.03 | 0.15** | **0.22*** |
| SupraMarginal | -0.03 | 0.15** | **0.25*** | 0.08 | 0.18** | **0.26**** |
| Angular | -0.06 | 0.11 | 0.14 | -0.01 | 0.18** | **0.22*** |
| Precuneus | 0.03 | 0.10 | **0.21*** | 0.03 | 0.08** | **0.25**** |
| Paracentral_Lobule | -0.01 | 0.07 | **0.21*** | 0.06 | 0.05 | **0.25*** |
| Caudate | 0.09 | 0.12** | **0.20** | 0.07 | 0.10** | **0.19** |
| Putamen | 0.03 | 0.16** | **0.24*** | 0.03 | 0.13** | **0.19** |
| Pallidum | 0.00 | 0.11** | **0.23*** | -0.03 | 0.13** | 0.10 |
| Thalamus | -0.02 | 0.03 | **0.20** | 0.02 | 0.02 | **0.18** |
| Heschl | 0.01 | 0.18** | **0.25** | -0.02 | 0.16** | **0.27**** |
| Temporal_Sup | -0.00 | 0.18** | **0.29**** | -0.02 | 0.18** | **0.27**** |
| Temporal_Pole_Sup | 0.04 | 0.17** | **0.23*** | 0.07 | 0.21** | **0.23*** |
| Temporal_Mid | -0.02 | 0.18** | **0.24*** | -0.04 | 0.17** | **0.25*** |
| Temporal_Pole_Mid | 0.03 | 0.17** | **0.26**** | 0.04 | 0.16** | **0.22*** |
| Temporal_Inf | -0.00 | 0.16** | **0.26**** | 0.04 | 0.11** | **0.28**** |

Underlined: significant correlations ($p < 0.05$) between local efficiency and mean IQ
Bold: FDR corrected significant correlations between local efficiency and mean IQ
* significant $r_{ph}$-g ($p < 0.05$)
** FDR corrected significant $r_{ph}$-g




**Supplemental Figure 6.2** – Development of FA-weighted local efficiency during adolescence. All 90 AAL regions are grouped in 6 large cortical and subcortical brain regions. Left and right hemispheres have the same color; dashed lines represent trajectory of local efficiency of regions in the right hemisphere, continuous lines represent left hemispheric regions.




**Supplemental Figure 6.3** – Presentation of the phenotypic (top, green), genetic (middle, pink), and unique environmental (bottom, blue) correlations between local efficiency and IQ at wave 1, 2, and 3. Dark colors indicate significance at $p < 0.05$; light colors indicate FDR-corrected significance. Size of the sphere is the absolute correlation strength; for the rph-e, the correlations are negative at wave 2, mainly positive at wave 3 (one region reached significance at $p < 0.05$; indicated in orange). Although there was a high genetic contribution to the correlation between IQ and local efficiency at age 13 (wave 2), the negative unique environmental influences led to a small phenotypic correlation. At age 18 (wave 3), the negative unique environmental correlation was not present, and the phenotypic correlation comes to bloom.

**Supplemental Table 6.2** – Heritabilities of local efficiency at all three measurements (M1, M2, M3); and the genetic correlation between local efficiency of measurements 1 and 2 (M1-2), and measurements 2 and 3 (M2-3).

| | Left | | | | | Right | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Broad heritability | | | Rg | | Broad heritability | | | Rg | |
| | M1 | M2 | M3 | M1-2 | M2-3 | M1 | M2 | M3 | M1-2 | M2-3 |
| Precentral | 0.43 | 0.20 | 0.39 | **0.76** | **0.94** | 0.11 | 0.19 | 0.17 | **0.76** | **0.85** |
| Frontal_Sup | 0.32 | 0.24 | 0.49 | **0.88** | **0.80** | 0.07 | 0.12 | 0.33 | 0.78 | **0.83** |
| Frontal_Sup_Orb | 0.25 | 0.21 | 0.44 | 0.69 | 0.59 | 0.07 | 0.08 | 0.30 | 0.42 | 0.55 |
| Frontal_Mid | 0.43 | 0.17 | 0.38 | 0.72 | 0.66 | 0.00 | 0.06 | 0.10 | 0.71 | 0.85 |
| Frontal_Mid_Orb | 0.31 | 0.17 | 0.33 | 0.67 | 0.81 | 0.14 | 0.13 | 0.15 | 0.80 | 0.96 |
| Frontal_Inf_Oper | 0.59 | 0.21 | 0.37 | 0.65 | **0.82** | 0.13 | 0.07 | 0.17 | 0.64 | 0.72 |
| Frontal_Inf_Tri | 0.23 | 0.10 | 0.32 | 0.54 | 0.63 | 0.25 | 0.09 | 0.15 | 0.48 | 0.86 |
| Frontal_Inf_Orb | 0.12 | 0.08 | 0.22 | 0.18 | 0.45 | 0.09 | 0.10 | 0.20 | 0.19 | 0.39 |
| Rolandic_Oper | 0.56 | 0.24 | 0.38 | **0.67** | **0.95** | 0.06 | 0.16 | 0.37 | 0.55 | **0.77** |
| Supp_Motor_Area | 0.44 | 0.19 | 0.40 | 0.65 | **0.92** | 0.15 | 0.18 | 0.36 | **0.78** | **0.66** |
| Olfactory | 0.22 | 0.07 | 0.29 | 0.38 | 0.65 | 0.02 | 0.04 | 0.30 | 0.15 | 0.28 |
| Frontal_Sup_Medial | 0.25 | 0.25 | 0.52 | 0.64 | **0.80** | 0.10 | 0.16 | 0.40 | 0.53 | **0.84** |
| Frontal_Med_Orb | 0.15 | 0.16 | 0.36 | 0.75 | **0.85** | 0.04 | 0.05 | 0.41 | 0.43 | 0.67 |
| Rectus | 0.23 | 0.16 | 0.39 | 0.60 | **0.81** | 0.07 | 0.10 | 0.20 | 0.17 | 0.39 |
| Insula | 0.29 | 0.15 | 0.31 | 0.61 | 0.66 | 0.18 | 0.12 | 0.22 | 0.74 | **0.85** |
| Cingulum_Ant | 0.22 | 0.17 | 0.42 | 0.55 | 0.36 | 0.12 | 0.12 | 0.45 | 0.69 | **0.66** |
| Cingulum_Mid | 0.34 | 0.26 | 0.41 | **0.87** | **0.64** | 0.24 | 0.19 | 0.30 | **0.87** | **0.70** |
| Cingulum_Post | 0.27 | 0.25 | 0.46 | **0.90** | 0.66 | 0.32 | 0.28 | 0.27 | **0.87** | **0.55** |
| Hippocampus | 0.30 | 0.30 | 0.32 | 0.77 | 0.62 | 0.07 | 0.14 | 0.12 | 0.58 | **0.67** |
| ParaHippocampal | 0.31 | 0.16 | 0.19 | -0.04 | 0.71 | 0.03 | 0.08 | 0.24 | 0.68 | 0.74 |
| Amygdala | 0.34 | 0.07 | 0.18 | 0.16 | 0.55 | 0.03 | 0.10 | 0.24 | 0.66 | 0.59 |
| Calcarine | 0.39 | 0.38 | 0.39 | **0.89** | **0.60** | 0.40 | 0.24 | 0.16 | **0.73** | 0.67 |
| Cuneus | 0.39 | 0.41 | 0.40 | **0.90** | **0.80** | 0.29 | 0.21 | 0.07 | **0.81** | 0.69 |
| Lingual | 0.31 | 0.28 | 0.40 | **0.89** | **0.78** | 0.00 | 0.02 | 0.07 | **0.93** | **0.76** |
| Occipital_Sup | 0.43 | 0.39 | 0.30 | **0.79** | **0.87** | 0.20 | 0.21 | 0.07 | **0.85** | **0.76** |
| Occipital_Mid | 0.30 | 0.40 | 0.42 | 0.64 | **0.72** | 0.31 | 0.07 | 0.05 | 0.51 | **0.87** |
| Occipital_Inf | 0.20 | 0.28 | 0.32 | 0.51 | 0.70 | 0.21 | 0.08 | 0.06 | 0.57 | 0.78 |
| Fusiform | 0.34 | 0.16 | 0.27 | 0.43 | 0.40 | 0.01 | 0.07 | 0.05 | 0.71 | 0.80 |
| Postcentral | 0.34 | 0.19 | 0.36 | **0.79** | **0.79** | 0.17 | 0.17 | 0.28 | **0.82** | **0.83** |
| Parietal_Sup | 0.26 | 0.33 | 0.48 | **0.73** | 0.48 | 0.13 | 0.15 | 0.06 | **0.80** | **0.59** |
| Parietal_Inf | 0.41 | 0.15 | 0.47 | 0.66 | 0.65 | 0.17 | 0.16 | 0.14 | **0.82** | **0.81** |
| SupraMarginal | 0.41 | 0.18 | 0.39 | 0.73 | **0.83** | 0.03 | 0.11 | 0.13 | **0.83** | **0.78** |
| Angular | 0.37 | 0.29 | 0.51 | **0.71** | **0.57** | 0.10 | 0.13 | 0.05 | **0.83** | **0.84** |
| Precuneus | 0.35 | 0.32 | 0.35 | **0.91** | **0.73** | 0.25 | 0.21 | 0.20 | **0.91** | **0.73** |
| Paracentral_Lobule | 0.42 | 0.26 | 0.33 | **0.94** | **0.95** | 0.34 | 0.22 | 0.20 | **0.97** | **0.79** |
| Caudate | 0.34 | 0.10 | 0.26 | 0.01 | 0.51 | 0.06 | 0.12 | 0.30 | 0.72 | **0.91** |
| Putamen | 0.41 | 0.12 | 0.33 | 0.65 | 0.77 | 0.06 | 0.08 | 0.23 | **0.87** | **0.87** |
| Pallidum | 0.41 | 0.19 | 0.35 | **0.86** | **0.90** | 0.24 | 0.17 | 0.48 | **0.82** | **0.65** |
| Thalamus | 0.42 | 0.18 | 0.19 | **0.92** | 0.75 | 0.33 | 0.18 | 0.35 | **0.95** | 0.66 |
| Heschl | 0.39 | 0.14 | 0.31 | 0.42 | 0.66 | 0.20 | 0.12 | 0.12 | 0.42 | **0.71** |
| Temporal_Sup | 0.50 | 0.16 | 0.44 | 0.67 | 0.70 | 0.20 | 0.11 | 0.07 | **0.68** | **0.93** |
| Temporal_Pole_Sup | 0.29 | 0.12 | 0.29 | 0.42 | 0.79 | 0.10 | 0.17 | 0.36 | 0.34 | **0.72** |
| Temporal_Mid | 0.48 | 0.20 | 0.50 | **0.75** | **0.71** | 0.33 | 0.15 | 0.16 | **0.75** | **0.85** |
| Temporal_Pole_Mid | 0.21 | 0.09 | 0.29 | 0.18 | 0.52 | 0.06 | 0.15 | 0.33 | 0.47 | **0.88** |
| Temporal_Inf | 0.43 | 0.16 | 0.41 | 0.63 | **0.86** | 0.03 | 0.10 | 0.09 | **0.85** | **0.94** |

All estimates of broad heritability were larger than zero (based on 95%CI)
Underlined: Rg is significantly ($p < 0.05$) different from zero
Bold: Rg is FDR corrected significantly different from zero

*Know why the nightingale sings*
*Is the answer to everything*

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Nightwish, *Know why the nightingale sings?* (1997)
Lyrics by Tuomas Holopainen

Chapter 7

# Summary & Discussion

**OVERVIEW**

Adolescence is a period of major changes in hormone levels, cognition, physical development, and brain development. The aim of this thesis was to study the development of the adolescent brain and how hormone levels and intelligence are related to that development. This was done with data from the BrainSCALE cohort, a longitudinal study with up to 3 assessments in twins and their older siblings from late childhood to late adolescence. The twin design allows for the disentanglement of genetic and environmental influences on the traits of interest. In this chapter, a summary per chapter is provided, followed by a discussion on the main findings. In short, the outcomes of the studies in this thesis can be summarized as follows:

- Reproductive hormone levels increase 2- to 9-fold from late childhood to early adolescence, and these levels are related to secondary sexual characteristics and structural brain development.
    - These traits and their correlations are largely explained by genetic factors, but environmental influences also play a role; the correlations between hormone levels and brain structure are driven by environmental factors.
- From late childhood to early adolescence, changes in the communication capacity of the structural brain network go hand in hand with changes in intelligence.
- During adolescence, the correlation between communication capacity of the structural brain network and intelligence increases.
    - This correlation is influenced by genetic factors; genes explain up to 87% of the observed correlation in late adolescence.
- Adolescent development of the communication capacity of the structural brain network follows a cubic pattern and is related to intelligence.
    - Communication capacity is influenced by genetic factors; heritability up to 66%.



**SUMMARY**

In this dissertation, I have studied changes in hormone levels, cognition and brain structure in a longitudinal design with 3 assessments in twins and their non-twin siblings to allow for the disentanglement of the influences of genes and environment on these developmental changes. The procedure of data collection for this project and in particular for the third measurement is described in **Chapter 2**. In Chapters 3 and 4, I analyzed data collected at ages 9 and 12 of the twins. In Chapter 5, twins and their siblings were included, resulting in a mean age of 10 and 13. In Chapter 6, all three assessments were included (mean age 10, 13, and 18 years for the twins and their siblings).

Between ages 9 and 12 years, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol and testosterone levels in boys and girls showed a 2- to 9-fold increase (**Chapter 3**). Hormone levels at age 9 in boys and girls were related to hormone levels and secondary sexual characteristics at age 12. Hormone levels and secondary sexual characteristics were both under influence of genes, as was the correlation between hormone levels at

age 9 and secondary sexual characteristics at age 12. An exception was seen in girls where a common environmental factor influenced the relation between estradiol levels at age 9 and stage of breast development at age 12. When comparing the heritability of hormone levels between the sexes, heritability estimates were higher in boys than in girls. Another difference between boys and girls was that LH was predictive of secondary sexual characteristics in girls whereas FSH was more predictive in boys. Both findings suggest that hormone levels act differently in boys and girls during early puberty.

Next, the relation between hormonal development and brain development was explored. **Chapter 4** demonstrated that in girls, changes in FSH levels between age 9 and 12 were positively related to change in gray matter density, and at age 12, estradiol was negatively related to gray matter density. No significant associations between hormone levels and gray matter density were found in boys. The relations between hormone levels and gray matter density or change in gray matter density were driven by environmental factors. These findings further illustrate the different developmental windows in boy and girls, as both physical development (Mul et al., 2001) and brain maturation (Raznahan et al., 2010) are delayed in boys when compared to girls. Although physical and brain development appear go hand in hand, it remains an open question whether FSH and estradiol cause the changes in gray matter density directly or whether the relation is indirect via an underlying third source that triggers both pubertal development in hormone levels and brain development.

**Chapters 5 & 6** focused on the development of the white matter network and reported the influence of intelligence on network development in typical adolescents. **Chapter 5** showed that changes in network efficiency (processing or communication capacity of the brain) were related to changes in IQ (intelligence quotient). Between ages 10 and 13, FA-weighted network efficiency increased on a brain wide level, with the largest increases in the posterior part of the brain. In addition to (developmental) changes in white matter network efficiency, teenagers also showed changes in IQ: one in six participants had a decrease or increase of more than one standard deviation (i.e. more than 15 IQ points) over a period of 3 years (between age 10 and 13 years). Interestingly, those participants with most pronounced increases in IQ also showed higher increases in global and local FA-efficiency. This positive correlation between change in IQ and change in local efficiency was present in frontal and temporal regions. Participants who lost IQ points showed a small decrease or remained stable in network efficiency over the 3-year interval; participants who gained IQ points showed an increase of local efficiency in these regions.

The development of streamline count-weighted network efficiency was also investigated. Streamline count refers to the number of lines that can be constructed between two regions; sometimes also referred to as fiber count. During the 3-year interval, both increases (frontal and occipital areas) and decreases (subcortical, temporal, and parietal regions) in local efficiency took place; resulting in a net-decrease of streamline count-weighted global efficiency. Changes in local streamline count-weighted efficiency were negatively related to changes in IQ. Clearly, the adolescent streamline count-weighted network reflects a different aspect of the white matter network than adolescent FA-weighted network.

Both FA- and streamline count-weighted local and global efficiency were for a large part influenced by genes, and a stable genetic factor influenced global and local efficiency of the network at both measurements. The correlation between IQ and local FA-efficiency was for a

large part driven by common genes. Factors influencing the changes in global or local FA network efficiency or changes in IQ could not be disentangled. No significant correlations between streamline-weighted network efficiency and IQ were found. In several regions, changes in local streamline count-weighted network efficiency were driven by genetic factors.

**Chapter 6** illustrated that during puberty, development of FA-weighted network efficiency could be characterized as a cubic function with age, with an increase in local and global efficiency from age 10 to 13, followed by a decrease until around age 20, after which efficiency increased again. Moreover, the development of the teenage network was related to IQ. On a group level, network efficiency decreased between ages 13-18. However, participants with a high IQ showed stable network efficiency in this age span. This was seen on a global as well as a local level. The correlation between network efficiency and IQ seems to be a process of adolescent development: whereas there was no correlation between local or global efficiency and IQ at age 10 and only a small correlation in a few regions at age 13, there was a brain wide, FDR-corrected significant correlation between local efficiency and IQ at age 18.

Local and global FA-network efficiency at age 18 were partly influenced by genes. The correlation between IQ and local FA-efficiency was to a large extent driven by genes that influence both FA-efficiency and IQ. This genetic association was also present at age 13, but interestingly, at age 13, a unique environmental factor had a negative influence on the correlation between network efficiency and IQ, resulting in small phenotypic correlations. This environmental factor was not present at 10 or 18 years.

## DISCUSSION

### *Cognitive development: changes in IQ?*

The considerable change in IQ observed between ages 10 and 13 in some teenagers may seem surprising. After all, the assessment of IQ takes age into account and IQ is assumed to be stable throughout life (Deary et al., 2000). Indeed, the majority of the teenagers in our cohort had a stable IQ over the 3 measurements, but 12% showed a decrease or increase of more than one standard deviation (15 IQ points) after three years, and 13% showed change of more than one standard deviation after 8 years. It has to be noted that a decrease in IQ does not necessarily mean that a participant performed worse on the task itself. In fact, often they performed the task better than 3 years before, however, relative to their peers task performance was not as good as 3 years ago. In addition, at all three assessments, a different set of IQ subtests was used (full scale WISC at assessment 1, six WISC subtasks at assessment 2, four WAIS subtasks at assessments 3), which could also account for individual longitudinal variance.

Several studies report changes in IQ during adolescence (Burgaleta et al., 2014; Ramsden et al., 2011; Waber et al., 2012). Because these studies also found a relation between changes in IQ and changes in the brain (Burgaleta et al., 2014; Ramsden et al., 2011), it seems unlikely that these findings are simply due to measurement errors. As discussed by Waber et al. (2012) and already considered in mid-twentieth century by Bayley (1949), it is likely that the underlying cause of these changes lies in individual differences of developmental pace. On a group level IQ was stable over the three measurements. The 95% confidence interval

of the change in IQ was -2.97 to -0.46 between age 10 and 13, and 0.71-3.55 between age 13 and 18. The correlations between the IQ measurements were 0.72 (between age 10 and 13) and 0.77 (between age 13 and 18), which is comparable to test-retest correlations over intervals of similar length in other cohorts (Truscott et al., 1994; Watkins and Smith, 2013).

Changes in IQ in the typical teenage population are not only an interesting finding in light of normal teenage cognitive development and teenage cognitive development in relation with brain development, but also in the light of the onset of psychiatric disorders. Several disorders have their onset during puberty (Kessler et al., 2007; Paus et al., 2008) and for some psychiatric illnesses, a drop in IQ can serve as a marker for disease onset or liability (Hedman et al., 2013; Khandaker et al., 2003; Mesholam-Gately et al., 2009). Hence, it is important to know that fluctuations in IQ also occur in the typical population.

***Brain development***

The main aim of this thesis was to get information on the development of the white matter network of the teenage brain. In addition, possible factors like pubertal hormones and cognitive functioning that could influence brain development were examined. Concerning white matter development, a profound increase of FA-weighted efficiency was found in early puberty, followed by a plateau or decrease in efficiency in mid puberty. While literature shows that white matter volume and FA increases until adulthood (Kochunov et al., 2012; Lebel et al., 2012; Lebel and Beaulieu, 2011), a stable or decrease in FA in mid puberty is also in agreement with the literature (Achterberg et al., 2016; Miller et al., 2012; Simmonds et al., 2014). The small decrease in FA-weighted efficiency is probably overseen by studies that explore a broad age range, and may reflect a period within adolescence entailing specific reorganization of the brain. This notion is supported by higher genetic influences on FA in left inferior and middle frontal gyri in teenagers compared to adults, suggesting local and temporary developmental mechanisms that influence FA (Chiang et al., 2011b). In our study (Chapter 6), heritability of FA-weighted network efficiency was lower at age 13 compared to age 10 and 18.

A change in efficiency can be caused by a change in edge weights (in our case FA or streamline count) and/or a change in distribution of weights and/or a change in binary topology. A decrease in local efficiency means that the direct neighbors of that node are less strongly interconnected (see Figure 1.1 on page 8; one has to keep in mind that a direct neighbor is not per se spatially close, but 'one edge apart'). A change in local efficiency thus refers to an increase or decrease in the interconnection of a local brain area with other brain areas via about several white matter bundles; it is a summary statistic. A decrease in efficiency can also co-occur with an increase in FA: it depends on which edges increase and which do not, leading to a change in weight distribution. Future studies are needed to determine which bundles had the strongest influence on the change in network efficiency. Since changes in network characteristics are directly or indirectly due to changes in edge weights, in the following paragraph mechanisms underlying changes in FA are considered. Factors influencing streamline counts are discussed in the next section.

*Mechanisms underlying changes in FA*

FA of the white matter is considered to reflect myelination, axon diameter, axon alignment,

and axon density (how many axons cross one voxel, thus also reflecting how much water is in between the axons); visualized in Figure 7.1 (Beaulieu, 2002). During puberty, myelin thickness as well as axon diameter are thought to increase (Paus, 2010a). It seems unlikely that at this age new axonal pathways are formed on a scale that can be measured, but it is possible that axons lie closer together. Another possible contributing factor to a decrease in FA is the occurrence of crossing fibers. The majority of the white matter voxels contain crossing fibers (estimates range from 63 to 90%; Jeurissen et al., 2013). When bundles in primary directions do not change while the secondary direction becomes more myelinated, FA of the voxel containing these crossing fibers will decrease. In our analyses, FA of a bundle was determined by mean FA over the entire length of the bundle. Localized changes due to crossing fibers thus influence FA of the whole bundle.

On a synaptic scale, it has been shown that new dendritic spines can evolve within one hour after onset of motor training in rats (Xu et al., 2009). In humans, changes in the white matter can be detected with MRI scans after six or more weeks of learning a new task or skill. Skill learning (reviews by (Taubert et al., 2012; Valkanova et al., 2014; Zatorre et al., 2012)) and physical exercise (Svatkova et al., 2015) are mostly associated with an increase in FA. However, there are also studies in rats (Blumenfeld-Katzir et al., 2011) and humans (Taubert et al., 2010) that report a localized decrease in FA after a training paradigm. The decreases in FA were local and accompanied by increases in FA in other regions. In rats, it was found that changes in FA after 5 days of training were related to an increased immunoreactivity and a number of astrocytic processes (Blumenfeld-Katzir et al., 2011). This indicates that learning can be accompanied by decreases in FA and that neuronal factors besides myelin influence observed changes in FA. On the other hand, the neuronal factors that influenced the change in FA in rats may be related to only a short-term change FA, which leads us to the question what governs long term changes in FA. (Tuch et al., 2005) did not investigate training-in-



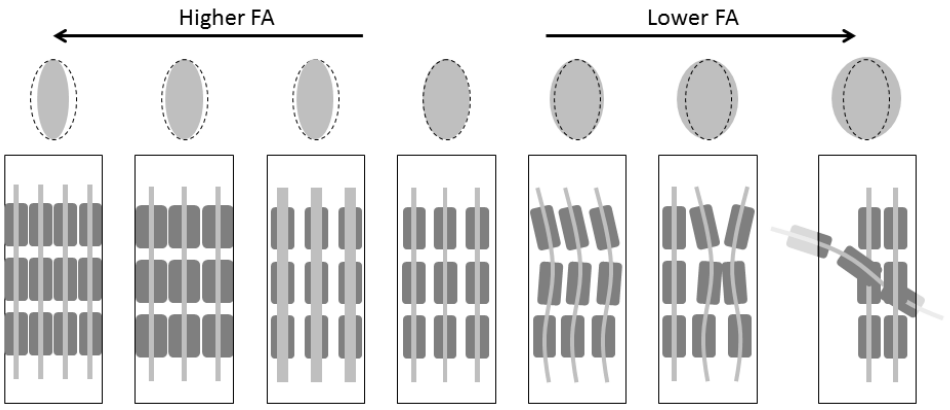


**Figure 7.1** – FA is influenced by packing density of fiber tracts, alignment of the fibers, axonal diameter, and myelination. Axons are reflected by light gray lines, myelin by dark gray rectangles surrounding the axons. When taking the middle example as a reference (dashed black oval), FA will be higher (thus a more elongated ellipsoid) with increased packing density, myelination and axonal diameter (left hand side). FA will be lower (more sphere like) with less-smooth alignment and crossing fibers (right side).

duced effects, but whether variance in reaction times could be explained by variance in FA of projection and association pathways supporting visuospatial attention. They report both negative and positive correlations between FA with reaction times. Similarly, Schmithorst and Wilke (2002) found lower FA of the corona radiata and internal capsule in musicians compared to non-musicians, and a higher FA in the genu of the corpus callosum. These relations between lower FA and 'better' skill are likely attributable to local rearrangements and/or crossing fibers.

It can be concluded from the above two paragraphs that the interpretation of changes in FA is not straightforward, and, that higher FA is not always for the better. Likewise, network efficiency is not always positively associated with functioning. For example, although lower efficiency has been related to several diseases (Bassett and Bullmore, 2009), a higher level of local efficiency has been related to autism (Courchesne et al., 2005) and ADHD (Cao et al., 2014; Konrad and Eickhoff, 2010) – two typical developmental disorders.

Developmental changes of structural network efficiency over the lifespan have mainly been investigated using streamline count as edge weight. One study examined the FA-weighted network efficiency in old age (Wen et al., 2011). Although streamline count is based on FA and thus related to FA, it is not the same (see next section). Previous literature reports that both increases and decreases in local streamline-weighted efficiency occur between 12 and 30 years (Dennis et al., 2013), and in adulthood (19-85yr) (Gong et al., 2009). Other studies found an increase in (weighted with inverse apparent diffusion coefficient (ADC); the magnitude of diffusion) global efficiency over the ages 2–18 (Hagmann et al., 2010) and a decrease in local and global streamline-weighted efficiency between the ages 4 and 40 (Lim et al., 2015). Our findings add to these reports that during early puberty (between 10 and 13 years), streamline count weighted efficiency increased in frontal and occipital areas, and decreased in subcortical, temporal, and parietal regions (Chapter 5). These changes were accompanied by a strong increase in white matter integrity and FA-weighted network efficiency. During mid-puberty, FA-weighted network efficiency decreased a little.

Additionally, our findings also provide evidence that developmental pace and shape, and the amount of genetic influences on FA-weighted local efficiency differ across brain regions. Future longitudinal follow-up studies of this cohort are needed to learn how FA-weighted efficiency develops after age 18. The white matter network is suspected to change at least until mid-30s because FA still increases until the third decade of life (Lebel et al., 2012; Yap et al., 2013) and white matter volume has been found to increase till age 45 (Hedman et al., 2012). As suggested, longitudinal studies are more likely to pick up fast changing developmental patterns that occur over a few years than cross-sectional studies spanning a decade or more.

*Streamline-weighted networks; how are they different from FA-weighted networks?*

Results presented in Chapter 5 indicate that during early adolescence, streamline-count weighted networks show a different aspect of the white matter network than FA-weighted networks. Streamline-count refers the number of lines that can be constructed between two regions. Although it may appear as if the number of streamlines is a proxy for the number of axonal connections, it cannot be interpreted as such: voxel size and number of seed points per voxel play a role in the detected number of streamlines. Because FA is used as a guide for the reconstruction process of streamlines, streamline-count reflects density of fiber tracts,

alignment of the fibers, axonal diameter, and myelination as well. Thus, factors influencing changes in streamline count during puberty can be the same factors as mentioned above that influence FA. However, streamline count is more vulnerable to length, curvature and branching of the bundle (Jones et al., 2013). FA of a bundle is computed as the average over the entire bundle. As such, a patch of low FA will have a small influence on the FA of the bundle. A lower number of streamlines due to e.g. branching of the bundle will be a weakest link or bottleneck of the number of streamlines that go from region A to B, and thus influence the entire bundle.

Two studies employed streamline and FA based analyses on data from adult cohorts. They found both types of analyses to be in agreement for associations with age (Stadlbauer et al., 2012) and IQ (Li et al., 2009), thus contrasting our findings. It is possible, however, that the relation between FA and streamline count – and their associations with IQ – changes with brain maturation. Indeed, a recent longitudinal study showed that during late adolescence both streamline and FA weights locally increase and decrease throughout brain. However, there seemed to be a bias towards FA-increases in hub-to-hub connections whereas streamline-count showed both increases and decreases in these connections (Baker et al., 2015). Moreover, the authors report that streamline-count increased in frontal regions and decreased in subcortical regions, which is in agreement with our finding that streamline-weighted local efficiency increased in frontal regions and decreased in subcortical regions. More studies are needed to see if streamline-count and FA become more similar in terms of associations with IQ when participants grow older.

***Associations between IQ and brain development***

Our finding of an association between changes in IQ and changes in white matter network efficiency (Chapter 5) suggests a possible causal relation between the white matter network and IQ. After all, the brain is the seat of intelligence; the development of one feature may be related to the other. Of course, the relation will probably not be a simple causal relation. The brain and IQ are likely interconnected through a feedback loop where small changes in the brain (that cannot (yet) be picked up with MRI scans) could lead to enhanced learning, new synaptic connections, a higher IQ, which in turn could lead to specific environment seeking behavior, stronger connections, and so forth.

We also found that the white matter network of the brain and IQ become more strongly correlated during adolescence, and that this association is in part explained by genes common to both brain network efficiency and intelligence (Chapter 6). Several mechanisms could explain the increase of a (genetic) correlation between white matter network efficiency and IQ in mid adolescence. First, it can be hypothesized that gene expression triggered by education may depend on IQ and thus diverge structural brain network development to more efficient networks in smarter children. For example, rats showed upregulated mRNA expression of brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF) in the hippocampus a few days after learning a spatial memory task (Gómez-Pinilla et al., 1998; Kesslak et al., 1998). This shows that training or activating the brain could lead to (a cascade of) activation of genes that regulate long-term plasticity. A second hypothesis is that people with a higher IQ may have different genetic variants that directly of indirectly cause genes related to adolescent brain development to be more efficiently translated. For

instance, heritability of FA in the thalamus, the genu and posterior limbs of the internal capsule, and the superior corona radiate was higher in people with high IQ compared to people with lower IQ (Chiang et al., 2011b). In another paper by these same authors, it was shown that the association between FA and object assembly performance was positive in BDNF-Val carriers but around zero or negative in BDNF-Met carriers (Chiang et al., 2011a). A third hypothesis is that teenagers may follow their 'genetic drive' (based on their IQ) to an environment that will trigger specific developmental changes. For example, genes that are involved in (higher) IQ may also make a person more curious and interested in school-related work. This may allow for a longer sensitive period in individuals with a higher IQ as reflected by a higher common environmental influence on IQ during adolescence in teenagers with a higher IQ compared to teenagers with a lower IQ (Brant et al., 2013).

Our results show that although a genetic correlation between IQ and network efficiency was present at age 13, a negative unique environmental correlation seemed to counteract this genetic correlation between IQ and global and local efficiency, leading to a lower phenotypic correlation. We do not know what specific environment may have caused the negative correlation. In the Netherlands, children change from primary to secondary education around the age of 12, and thus change schools and peers. Possibly, the higher unique environmental variation of global and local efficiency at 13 reflects the effects of this new unique environment on individual variation of 'stage of brain development'. As a result, the genetic programmed stage of development (and thus the correlation with IQ) may be overruled by environmental effects. This environmental influence may also increase the variation in the pace of individual brain development, and thus partly account for the correlation between change in IQ and change in network efficiency between the ages 10 and 13 (Chapter 5) that was not observed between ages 13 and 18.

Environmental factors may also positively influence the relation between brain structure and intelligence as both IQ and brain structure can be influenced by environmental factors. IQ in childhood is highly correlated with IQ in old age suggesting a high level of stability throughout life (Deary et al., 2000). However, despite being overall stable, IQ can change in relation to the environment. Intellectual abilities of children adopted from a deprived (institutional) background often increase in their new, relatively enriched, environment (van IJzendoorn et al., 2005). This illustrates that childhood IQ has been shown to have a higher influence of the common environment than adult IQ (Plomin and Deary, 2015). Similarly, heritability of regional brain structure depends on both age and relative developmental timing of that brain region (Lenroot et al., 2009): regions that are not fully developed show a higher influence of environmental factors, allowing for environmental influences during adolescence. Together with our findings, this shows that both IQ and the brain are a work in progress and that they may influence each other.

Future research is needed to investigate the correlation between IQ and the white matter network – as well as other brain features, e.g. cortical thickness (Brouwer et al., 2014; Schnack et al., 2015) – after age 18 to determine if the correlation reaches a stable adult-like level around age 18, or if it decreases or continues to fluctuate throughout life. A study by Tamnes et al. (2011) found that the correlations between IQ and brain structure (cortical thickness, white matter volume, FA, MD) were stronger in teens (8-20 yr) compared to young adults (21-31yr). In addition, it would be of value to know how development of white

matter is related to development of gray matter in relation to IQ (cf. Bohlken et al., 2016b).

***Influence of hormones on typical brain development***

Because of our interest in the relation between brain development and hormonal development, I investigated the presence of an association between hormone levels and white matter network characteristics. Associations between testosterone levels and white matter integrity have often been reported in pubertal boys and young adults (Herting et al., 2012; Menzies et al., 2015; Peper et al., 2013; Peper et al., 2015; Perrin et al., 2008). Therefore, we expected to find at least a correlation with testosterone. However, no significant associations were found (unpublished data). Possibly, network analyses are too large scale to find significant correlations: brain wide network characteristics take all bundles in consideration, and local efficiency also comprises several connections. On the contrary, many previous studies report small regions on the white matter skeleton, or study specific regions or bundles. The next paragraph discusses existing literature on testosterone and white matter integrity or volume in pubertal boys and girls.

Although quite a few studies have reported a correlation between pubertal testosterone levels and white matter volume or integrity in boys (Herting et al., 2012; Menzies et al., 2015; Paus et al., 2010; Peper et al., 2015; Perrin et al., 2008), there are some discrepancies between these studies. Some report a positive association between testosterone and white matter integrity (Herting et al., 2012; Paus et al., 2010; Perrin et al., 2008), whereas others report a negative association (Menzies et al., 2015; Peper et al., 2015). In addition, some find a stronger association with MD than FA (Menzies et al., 2015) or the other way around (Herting et al., 2012). Literature is also not conclusive on the relation between testosterone and white matter integrity in girls. Some studies find a correlation with white matter integrity (Peper et al., 2015) whereas other do not (Herting et al., 2012; Peper et al., 2013). These inconsistencies could be due to selection of specific regions or bundles, and whole brain vs. regional analyses. In addition, the measurement of steroids differs between studies; saliva and serum testosterone levels may be highly correlated but interpretation and comparison between the two is complicated (Peper et al., 2011a). A final influencing factor could be minor differences in pubertal status: when participants are in different developmental windows, different associations may be present at that specific developmental period and in that region of the brain.

Chapter 4 reports that in girls, higher estradiol levels were related to a more mature brain (i.e., lower cortical gray matter density). Additionally, girls that increased the most in FSH levels also showed local increases in (sub-) cortical gray matter. These associations were partly driven by a (common or unique) environmental factor whereas no genetic influences on these associations could be found. We think an early pubertal marker, which triggers both these hormonal and brain changes, is the underlying cause of the correlations. The early pubertal marker could in turn be influenced by environmental factors like alcohol use (Davis et al., 2015; Dees et al., 2009; Dees et al., 2015; Peck et al., 2011; Richards and Oinonen, 2011), tobacco use (Davis et al., 2015; Peck et al., 2011), diet (Mervish et al., 2013; Sowers et al., 2006; Wolff et al., 2015), and father absence (Deardorff et al., 2002). Examples of early pubertal markers include gonadotropin-releasing hormone (GnRH), kiss-peptins that stimulate GnRH (Smith and Clarke, 2007) or other factors triggering the growth spurt

such as growth hormone, or insulin-like growth factors (Styne, 2003). Another promising candidate is the steroid hormone dehydroepiandosterone (DHEA) which has been shown to associate with cortical thickness, especially in the age range between 4 and 13 years (Nguyen et al., 2013b).

In our study, including measurements of 9 and 12 years old twins, significant associations between hormone levels and structural brain parameters were found only in girls, only for FSH and estradiol, and only in gray matter (Chapter 4). Probably, the boys in our cohort were too young to show enough variation in their hormone levels to find an association between hormones and the brain; results regarding testosterone levels at age 17 years await further analysis. Associations between testosterone and cortical thickness (Nguyen et al., 2013a; Nguyen et al., 2013b; Raznahan et al., 2010) or white matter (Herting et al., 2012; Menzies et al., 2015; Paus et al., 2010; Peper et al., 2015; Perrin et al., 2008) in boys have been found in other studies. Reports of correlations between LH and brain development are scarce. We found a correlation between LH and white matter volume in 9 year old boys and girls analyzed together (Peper et al., 2008), but not separately at age 9 or 12 (Chapter 4). No other studies have reported an association between LH and white matter during puberty. In young adults, a negative correlation between LH and MD of the fornix has been reported (De Bondt et al., 2013), suggesting the possibility of a relation between LH and white matter during pubertal development. However, the group size was small and participants with a natural cycle were combined with participants that used oral contraceptives, making interpretations tentative.

Future studies could look at the development of specific white matter bundles in relation to developmental increases in testosterone or other hormone levels. An interesting point of research are the cortico-subcortical connections. There is evidence from electro-encephalo-graphic (EEG) data and functional connectivity MRI data that testosterone plays a role in decreasing subcortical-cortical connectivity, and increasing connectivity between subcortical areas (Miskovic and Schmidt, 2009; Schutter and van Honk, 2004; Volman et al., 2011; van Wingen et al., 2010). In addition, recent work by Peper and colleagues suggest a role for testosterone in frontostriatal (Peper et al., 2013) and fronto-temporal-subcortical (Peper et al., 2015) white matter bundles mediating aggression in boys and girls (Peper et al., 2015) and impulsivity in boys (Peper et al., 2013). With our longitudinal data, future studies can look at the influence of testosterone on development of specific white matter bundles in relation to behavioral traits during mid and late adolescence. This can yield important new insights on the development of problem behavior in adolescents.

***Sex differences***

Although several studies report sex differences in FA during childhood and adolescence (Lebel and Beaulieu, 2011; Schmithorst et al., 2008; see also review by Ladouceur et al., 2012), no significant sex differences in FA-weighted efficiency were found in our studies. Boys had a larger streamline-weighted efficiency, which is most likely due to larger brain size of boys: they have thus more voxels, more starting points and more streamlines. One possibility for our null finding concerning FA-weighted efficiency is that the differences between boys and girls (at this age) are on a small or regional scale that is not picked up in our network approach. Here I give a short overview of sex differences in the brain during

puberty and adulthood.

Studies in adolescents often report that boys have higher FA, or that boys have a steeper developmental increase than girls do (Ladouceur et al., 2012). We found this pattern in the developmental trajectory of local FA network efficiency in only a few regions: boys tend to start with lower FA-weighted efficiency at age 10, show a steeper increase and end somewhat higher than girls do around age 18. Because the brains of girls develop earlier than boys (Raznahan et al., 2010; Raznahan et al., 2011), boys and girls of the same age can have different brain characteristics. As such, sex differences of brain development are difficult to interpret: they could either reflect increased sex differentiation or a temporary pubertal-timing induced difference (as discussed by Ladouceur et al., 2012). Already at birth, the male brain is larger than the female brain (also after co-varying body length or weight, Ankney, 1992; Witelson et al., 2006) and this continues into adulthood. Additionally, men tend to have a (relatively) larger white matter volume and higher FA than women (Menzler et al., 2011, review by Gong et al., 2011), although this may be related to brain size (Westerhausen et al., 2011). Thus, it seems reasonable that the sex differences in the brain that are observed during adolescence are not only due to a difference in the timing of brain development, but also reflect the start of increased sex differentiation of the brain (Paus, 2010b). In addition, studies by Perrin and Paus and colleagues suggest that white matter development may be governed by different mechanisms in boys and girls: white matter growth during adolescence in males is mainly influenced by increases of axonal caliber, whereas increased myelination plays a larger role in females (Paus and Toro, 2009; Perrin et al., 2009). Mechanisms by which sex differences in brain structure could be governed may also be the result of different behavior of genes in the sexes due to interaction of genes with sex-linked genes, and/or hormonal influences on gene expression and regulation (Weiss et al., 2006).

Interestingly, the correlation between IQ and FA-weighted local efficiency at age 18 was mainly driven by the girls in our cohort. A likely explanation is that girls start their pubertal development earlier than boys do – with regard to both secondary sexual characteristics and brain development. Possibly, the sex difference in manifestation of IQ in the brain at age 18 is the result of developmental timing. A fourth follow-up measurement in this cohort would be able to elucidate this question. An alternative explanation for our results is that men and women have different neuronal pathways to IQ (see review by Deary et al., 2010). It has been suggested that there is a higher association between IQ and white matter in women than in men (Gur et al., 1999; Haier et al., 2005), although there are also studies that find the opposite (Dunst et al., 2014). A study by Schmithorst (2009) reports a positive correlation between IQ and FA in girls, and a negative correlation in boys. Moreover, these correlations were present in the older children (12-18yr) and not in the younger children (5-11yr). In adults, positive correlations between cognitive functioning and FA were found in women, and negative correlations in men (Tang et al., 2010). This suggests that sex dependent correlations between IQ and white matter during adolescence could reflect the beginning of a differentiation between male and female 'intelligence paths in the brain'.

***Implications***

The studies described in this thesis cover different aspects of adolescent development. Future studies in health and psychiatric disorders can build on our findings. The finding of

different developmental trajectories depending on IQ implies that studying (aberrant) developmental trajectories of teenagers who are at high risk for psychiatric disorders, such as schizophrenia or depression, may be more informative and predictive than a brain scan at a single time point (Giedd et al., 2008; Moran et al., 2013; Rapoport and Gogtay, 2008; Shaw et al., 2010).

Another approach for studies that want to find markers for disease liability could be the combination of hormone levels and brain structure. Hormones influence behavior and the brain (Peper and Dahl, 2013) and hormone levels may be different in adolescents at low vs. high-risk for psychiatric disorders (Martel et al., 2009; van Rijn et al., 2011). As a result, different hormone levels could lead to different developmental trajectories or influence development of the healthy brain differently than the predisposed brain (cf. Peper et al., 2011b). Because we did not find a relation between hormone levels and white matter network topology, future studies may want to use a bundle- or voxelwise approach.

Heritability of network efficiency in adolescents was moderate to high. Structural and functional network efficiency is affected in several (heritable/genetic) psychiatric diseases (Bassett and Bullmore, 2009; Bohlken et al., 2016a; van den Heuvel and Fornito, 2014; van den Heuvel and Hulshoff Pol, 2010). Genetic mechanisms that influence network topology in the healthy population may also be involved in diseases that show aberrant network characteristics. The genetic link between brain structure and IQ may be involved in the onset of psychiatric disease. Recently, it was found that IQ, FA, and cortical thickness attribute to schizophrenia partly through a common genetic factor, as well as independently (Bohlken et al., 2016b). Understanding the environmental and genetic factors that influence hormone levels, white matter efficiency, intellectual functioning, and their relations is fundamental when looking for ways to optimize developmental trajectories for every individual.

*Methodological considerations*

Although the studies presented in this thesis were not specifically focused on the methodological aspects of studying brain development, the following findings deserve some consideration.

First, a broad age range during a period of rapid development like puberty may include a considerable variance. It is important to be aware of developmental or pubertal stages in addition to age that may increase the variance of the variable of interest.

A second point of consideration is the edge-weight of the structural brain networks: in early adolescence, different results were found when using streamline-weighted or FA-weighted networks (Chapter 5). This emphasizes the difference between two closely related white matter features. Hence, careful consideration is required when selecting edge weights, interpreting (developmental) changes in edge weights (see also Jones, 2010; Jones et al., 2013), and comparing studies that differ in network construction. Future studies are encouraged to examine both features in order to learn more about the developmental differences.

## CONCLUDING REMARKS

The aims of the studies in this thesis were to gain insight into seemingly very different processes of typical adolescent development. We conclude that changes in hormone levels, cog-

nition, and brain structure co-occur and are influenced by the same genetic and environmental factors. Adolescent-specific brain development is linked to puberty, and intellectual functioning is related to development of the white matter network. Genes play an important role in hormonal, physical, and brain development. This thesis provides new and important information for future studies to build on. It also invites new research to study the mechanisms that underlie the relation between IQ or hormones and brain development. Another question is how the brain continues its development after age 18. A fourth inclusion of the BrainSCALE participants would be most suitable to answer that question.

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# Appendices

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| DATUM | ONS KENMERK | UW BRIEF VAN | UW KENMERK |
|---|---|---|---|
| | NTR/brainscale | | |
| E-MAIL | TELEFOON | FAX | BIJLAGE(N) |
| s.c.swagerman@vu.nl | 020 598 5131 / 598 8827 | 020 598 8832 | informatiefolder |

Beste <<voornaam>>,

Een paar jaar geleden heb je meegedaan aan belangrijk onderzoek naar de ontwikkeling van het brein bij jongeren. Dit onderzoek werd uitgevoerd door Marieke, Jiska en Inge van het Nederlands Tweelingen Register (NTR) en het UMC Utrecht (UMCU).
Wij, Suzanne en Marinka, zijn de nieuwe onderzoekers op dit project en we zijn benieuwd hoe je hersenen de afgelopen jaren verder zijn ontwikkeld. Want hersenen blijven nog tot je 25e veranderen: het brein groeit nog steeds en de verbindingen en communicatie tussen hersengebieden veranderen. Zo worden sommige vaardigheden beter en leer je nieuwe aan: hierdoor wordt niet alleen je brein, maar ook je gedrag steeds volwassener. Wij willen gaan onderzoeken hoe deze ontwikkeling verloopt en hoe belangrijk de rol van erfelijke factoren is in deze processen: vandaar dat we je graag weer terug zien!

*Hoe ziet dit onderzoek er uit?*
Je komt een halve dag naar het UMC Utrecht. Daar maken we foto's van je hersenen in een MRI-scanner (je mag een dvd of cd meenemen). Ook doen we onderzoek naar geheugen, reactiesnelheid, ruimtelijk inzicht en het verwerken van emoties. Dit onderdeel is iets korter dan voorheen en bestaat voor het grootste deel uit een serie nieuwe computertesten. Ook meten we je lengte, gewicht en bloeddruk en vragen we je thuis urine en speeksel te verzamelen, daarin worden hormonen bepaald. In de folder kun je meer lezen over het onderzoek. Je kan ook op onze website (www.tweelingenregister.org/brainscale) kijken voor meer informatie, foto's en filmpjes over hersenontwikkeling.

*Wat krijg je als je mee doet?*
We vergoeden de reiskosten en je krijgt een cadeaubon ter waarde van €50. Bovendien krijg je een overzicht van je resultaten en een opname van je hersenen.
Over ongeveer twee weken nemen wij telefonisch contact op om uitleg te geven en een afspraak te maken. Als je na het lezen van deze brief en de informatiefolder nog vragen hebt, bel ons gerust of stuur een mailtje. We zijn heel blij als jullie weer mee willen doen!

Met vriendelijke groet,

Suzanne Swagerman, MSc. (NTR, tel: 020-598 5131, e-mail: s.c.swagerman@vu.nl) en
Marinka Koenis, MSc. (UMCU, tel: 088-755 3386, e-mail: m.m.g.koenis@umcutrecht.nl)

Mede namens:
Prof. dr. Hilleke Hulshoff Pol, prof. dr. Dorret Boomsma en dr. Rachel Brouwer

Aan de ouders / verzorgers van:
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| DATUM | ONS KENMERK | UW BRIEF VAN | UW KENMERK |
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Geachte ouder(s) en/of verzorger(s),

In de afgelopen jaren hebben u en uw kinderen meegedaan aan belangrijk onderzoek naar de ontwikkeling van het brein bij jongeren. Dit onderzoek werd uitgevoerd door Marieke, Jiska en Inge bij deelnemers van het Nederlands Tweelingen Register (NTR).

Wij zijn heel blij dat ook het NWO (Nederlandse Organisatie voor Wetenschappelijk Onderzoek) dit onderzoek van groot belang vindt en twee nieuwe onderzoekers heeft aangesteld om dezelfde tweelingen en hun broers en zussen uit te nodigen voor een tweede of derde meting. Tijdens de puberteit verandert de structuur van het brein en gaat het anders functioneren. Dit brengt veranderingen in gedrag en in cognitieve vaardigheden met zich mee. Een zeer belangrijke periode in de ontwikkeling naar volwassenheid, vandaar dat wij iedereen weer uitnodigen!

Net als de eerdere keren worden de hersenfoto's gemaakt in de MRI-scanner in het UMC Utrecht. Op dezelfde dag doen we ook het onderzoek naar hersenfuncties (geheugen, reactiesnelheid, ruimtelijk inzicht en het verwerken van emoties). Dit onderdeel is iets korter dan voorheen en bestaat voor het grootste deel uit een serie nieuwe computertesten. Bovendien worden lengte, gewicht en bloeddruk gemeten en bepalen we hormoonwaarden in ochtendurine en speeksel.
Aanwezigheid van de ouders op deze dag is niet noodzakelijk voor het onderzoek, maar u bent van harte welkom om mee te komen. Wel willen we u vragen enkele korte vragenlijsten in te vullen, die u per post kunt terugsturen. Het is uiteraard mogelijk om aparte afspraken te maken als het lastig blijkt om één afspraak met het hele gezin te maken. In de bijgesloten informatiefolder of op onze website (www.tweelingenregister.org/brainscale) leest u meer over het onderzoek.

Alle reiskosten worden vergoed. Als bedankje voor hun deelname krijgt iedereen een waardebon van €50,- en de resultaten van de testen en een foto van de hersenen.

Binnen twee weken nadat u deze brief heeft ontvangen neemt Suzanne of Marinka telefonisch contact op om verdere uitleg te geven en een afspraak te maken. Als u vragen heeft, kunt u uiteraard ook direct contact met ons opnemen.

Wij hopen iedereen opnieuw bij dit onderzoek te mogen verwelkomen!
Met vriendelijke groet,

Suzanne Swagerman, MSc. (NTR, tel: 020-598 5131, e-mail: s.c.swagerman@vu.nl) en
Marinka Koenis, MSc. (UMCU, tel: 088-755 3386, e-mail: m.m.g.koenis@umcutrecht.nl)

Mede namens:
Prof. dr. Hilleke Hulshoff Pol, prof. dr. Dorret Boomsma en dr. Rachel Brouwer

**Verzekering**

Deelname aan het onderzoek valt onder de aansprakelijkheidsverzekeringen van de VU en het UMCU. Deze verzekeringen dekken schade door dood of letsel die het gevolg is van deelname aan het onderzoek en die zich gedurende de deelname aan het onderzoek openbaart, of binnen vier jaar na beëindiging van de deelname aan het onderzoek. De schade wordt geacht zich te hebben geopenbaard wanneer deze bij de verzekeraar is gemeld.

De verzekering van het VU medisch centrum loopt bij Onderlinge Waarborg maatschappij Centramed B.A. Bij letselschade kunt u contact opnemen met Dhr. A.E. Santen op telefoonnummer 070-3017070. Deze verzekering vergoedt € 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 R. van Harten. Deze is te bereiken bij Van Lanschot Assurantien b.v. op telefoonnummer 073-6924762 of e-mail r.vanharten@vanlanschotchabot.com. De verzekering biedt een maximum dekking van € 450.000 per proefpersoon en € 3.500.000 voor het gehele onderzoek, en € 5.000.000 per jaar voor alle onderzoeken van dezelfde opdrachtgever. De dekking van specifieke schades en kosten is verder tot bepaalde bedragen beperkt. Dit is opgenomen in het Besluit verplichte verzekering bij medisch-wetenschappelijk onderzoek met mensen. Informatie hierover kunt u vinden op de website van de Centrale Commissie Mensgebonden Onderzoek: www.ccmo.nl.

Voor beide verzekeringen geldt dat uitgesloten van dekking is schade:

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

**Meer informatie**

Speciaal voor het huidige onderzoek is een website gemaakt: **www.brains4teens.nl**. Hier kan je informatie vinden over het onderzoek, hersenen, hersenfuncties en puberteit. Wil je meer weten over tweelingen, het NTR en het onderzoek dat gedaan wordt vanuit het NTR, surf dan naar www.tweelingenregister.org.

Heb je nog vragen over het onderzoek, neem contact op met een van de onderzoekers:
Suzanne Swagerman, MSc. (NTR) 020-598 5131 s.c.swagerman@vu.nl
Marinka Koenis, MSc. (UMCU) 088-755 3386 m.m.g.koenis@umcutrecht.nl





*onderzoek naar de ontwikkeling van brein en cognitie bij jongeren*

# BrainSCALE

# Informatie

Tweelingen en hun familieleden zijn belangrijk voor medisch en wetenschappelijk onderzoek: zo kunnen wetenschappers erachter komen in hoeverre verschillen in gedrag, gezondheid, hersenfuncties en denkvermogen worden beïnvloed door verschillen in erfelijke aanleg (de genen) of door leefomgeving.

Jij en je broers of zussen vormen een unieke groep die heeft meegewerkt aan eerder onderzoek van het Nederlands Tweelingen Register en het UMC Utrecht. Dankzij jullie medewerking aan het ***onderzoek naar de ontwikkeling van brein en cognitie bij jongeren*** is al veel bekend geworden over erfelijke aanleg van hersenontwikkeling en leervermogen bij kinderen. Dat jullie al een of twee keer eerder hebben meegewerkt hieraan is van onschatbare waarde voor de wetenschap. Bedankt hiervoor!

**Waarom wordt dit onderzoek herhaald?**

Het doel van het onderzoek is meer te weten te komen over hoe hersenen en hersenfuncties veranderen tijdens de ontwikkeling van jongere naar volwassene. Dit onderzoek helpt ons om te

begrijpen hoe de ontwikkeling van de hersenen verloopt en hoe het komt dat jongeren van elkaar verschillen in hun verstandelijke vermogens. Cognitieve vaardigheden hebben te maken met verstandelijke vermogens zoals: het opnemen en verwerken van kennis (leren), waarnemen, denken, taal, bewustzijn, geheugen, aandacht, concentratie en praktische vaardigheden. In deze folder noemen we al deze vaardigheden samen 'cognitie'.
Op je 16de jaar is je brein nog lang niet uitgegroeid (bij de meeste mensen pas rond hun 25e) en ook je hersenfuncties zijn zich nog aan het ontwikkelen. Als dezelfde jongeren meerdere keren meedoen aan dit onderzoek kunnen de veranderingen in ontwikkeling worden bestudeerd: bij wie gaat de groei van het brein snel? Bij wie niet?

**Waarom onderzoek bij tweelingen en hun broers en zussen?**

Dat mensen van elkaar verschillen in allerlei eigenschappen (gewicht, geheugen, leeftijd waarop ze in de puberteit komen) kan komen door verschillen in erfelijke aanleg, doordat ze opgroeien in verschillende gezinnen of omdat hun omgeving anders is (stad versus platteland bijvoorbeeld). Om hier onderzoek naar te doen zijn tweelingen en hun familieleden erg belangrijk. Gezinsleden hebben namelijk gedeeltelijk dezelfde omgeving (zoals voeding of leefstijl) en delen ook hun erfelijk materiaal (DNA). Eeneiige tweelingen hebben hetzelfde DNA, terwijl twee-eiige tweelingen net als broers en zussen gemiddeld voor 50% hetzelfde DNA hebben.
Door een- en twee-eiige tweelingen en hun broers en zussen met elkaar te vergelijken kan worden bepaald hoe genen en omgeving samenwerken en van invloed zijn op de ontwikkeling van hersenen en cognitie.

**Wat kun je verwachten bij het onderzoek?**

Het onderzoek duurt ongeveer een halve dag en vindt plaats in het UMC Utrecht. Het is niet noodzakelijk dat ouders meekomen naar het onderzoek, al mag het uiteraard wel. Aan ouders willen we vragen enkele korte vragenlijsten in te vullen. Iedere deelnemer ontvangt een reiskostenvergoeding en een cadeaubon (zie hieronder).

*Onderzoek naar cognitieve vaardigheden*

Er wordt een aantal testen gedaan op de computer waarmee wordt gekeken naar onder andere geheugen, reactiesnelheid, ruimtelijk inzicht en het verwerken van emoties. Sommige onderdelen kun je herkennen van vorige onderzoeken, maar het grootste gedeelte van de cognitieve vaardigheden meten we met een nieuwe serie computertesten. Deze kun je grotendeels zelfstandig uitvoeren, maar er is altijd iemand aanwezig voor aanwijzingen of vragen. In totaal duurt dit gedeelte twee uur, dit is inclusief pauze.

*MRI-scanner*

De "foto's" van je hersenen worden gemaakt in een MRI-scanner, in totaal lig je daar zo'n 50 minuten in. Het is belangrijk dat je tijdens het maken van de scans zo stil mogelijk blijft liggen. Tijdens het eerste gedeelte van de scan kan je (zelf meegebrachte) muziek luisteren of een dvd kijken, maar tijdens de laatste 10 minuten mag je niets doen en hou je je ogen dicht: zo wordt gemeten wat er in de hersenen gebeurt wanneer ze niet actief bezig zijn.
Bij MRI wordt gebruik gemaakt van een magneetveld en radiogolven, wat niet schadelijk is voor de gezondheid. Omdat je voor de scan in een sterk magnetisch veld ligt moet je daarvoor in een speciale kleedruimte alles waar metaal in zit afdoen (zoals sieraden, haarclipjes, kledingstukken: persoonlijke eigendommen kunnen veilig worden opgeborgen).
Je krijgt van ons een mooie broek zonder metaal erin om aan te trekken. Wist je dat in sommige make-up ook metaal zit? Aangezien je ogen dicht bij de hersenen liggen zorgt die make-up er voor dat de hersenscans niet helemaal scherp zijn. Vandaar dat wij zorgen voor spullen waarmee je je make-up kan verwijderen voordat je de scanner in gaat.
Een beugel? Helaas kun je hiermee niet in de MRI-scanner. Misschien ben je om deze reden de vorige keer niet in de scanner geweest: dat maakt nu niet uit, je kunt gewoon weer meedoen als je beugel er uit is. Een draadje achter de tanden is geen probleem.

*Hormoononderzoek en lichamelijk onderzoek*

Om de informatie over hersenen en hersenfuncties goed te kunnen begrijpen is het belangrijk te weten hoe ver je in de puberteit bent. Daarvoor willen we je vragen thuis ochtendurine en speeksel te verzamelen. Hierin worden hormonen gemeten die het lichaam aanmaakt vanaf de start van de puberteit. Ook maak je thuis een monduitstrijkje voor de analyse van erfelijk materiaal (DNA).
Het lichamelijk onderzoek naar puberteitskenmerken maakt niet langer deel uit van het onderzoek: we vragen je nu om zelf aan te geven op een formulier hoe ver je in de puberteit bent. Als laatste meten we je gewicht, lengte en bloeddruk.

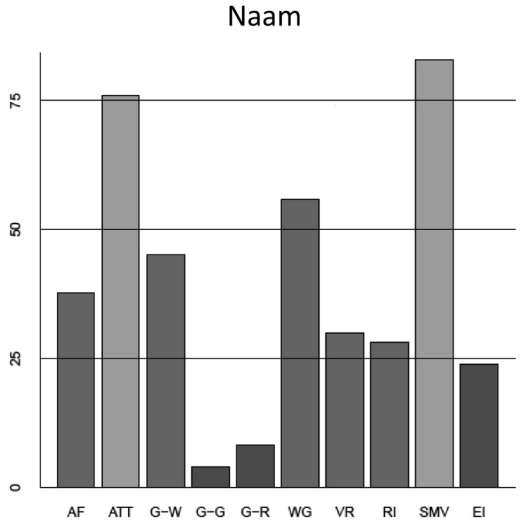
**Wat verder van belang is om te weten**

*Privacy*

Alle gegevens die met dit onderzoek worden verzameld worden vertrouwelijk behandeld. De gegevens worden bewaard en verwerkt onder een nummer en dus niet onder je naam of andere persoonlijke gegevens. Een paar andere mensen kunnen wel de persoonlijke gegevens zien. Deze mensen controleren of het onderzoek goed en betrouwbaar is. In de Wet bescherming persoonsgegevens is vastgelegd hoe dit moet gebeuren. Mensen die de gegevens kunnen zien, zijn bijvoorbeeld: het onderzoeksteam, de toetsingscommissie, de veiligheidscommissie die het onderzoek in de gaten houdt, de Inspectie voor de Gezondheidszorg.
De gegevens van dit onderzoek worden gecombineerd met de eerder verzamelde gegevens. Zo kijken we bijvoorbeeld of hersengroei samenhangt met geboortegewicht. Resultaten kunnen in het kader van (inter)nationale samenwerking worden gedeeld met andere onderzoekers. In publicaties zijn geen namen terug te vinden.

*Praktische informatie*

- ✓ Voor je deelname aan het onderzoek ontvang je een waardebon van €50,-
- ✓ Daarnaast worden de gemaakte reiskosten vergoed
- ✓ Het onderzoek duurt ongeveer 5 uur
- ✓ Voor eten en drinken wordt gezorgd
- ✓ Deelname is vrijwillig en je kan je op elk moment, ook na ondertekening van het toestemmingsformulier, zonder opgave van reden uit het onderzoek terugtrekken. Dit heeft geen nadelige gevolgen voor je verdere relatie met het Nederlands Tweelingen Register of wanneer je voor andere behandelingen het UMCU bezoekt
- ✓ Mochten er naar aanleiding van het MRI-onderzoek bevindingen zijn waarvoor medisch handelen noodzakelijk wordt geacht, dan worden de betrokkene en de huisarts hiervan op de hoogte gestel
- ✓ Als je een deskundige wilt spreken die niet betrokken is bij het onderzoek om onafhankelijke informatie te krijgen, dan kan je contact opnemen met Petra Zwijnenburg, arts (telefoon: 020-4440150/-3389, mail: p.zwijnenburg@vumc.nl)
- ✓ Je ontvangt de belangrijkste resultaten van het onderzoek en een foto van je hersenen




| | | |
|---|---|---|
| AF = Abstractie en Flexibiliteit | G-R = Geheugen 3D Figuren | RI = Ruimtelijk Inzicht |
| ATT = Aandacht | WG = Werkgeheugen | SMV = Senso-Motorische Verwerkingssnelheid |
| G-W = Geheugen Woorden | VR = Verbaal Redeneren | EI = Emotie Identificatie |
| G-G = Geheugen Gezichten | | |

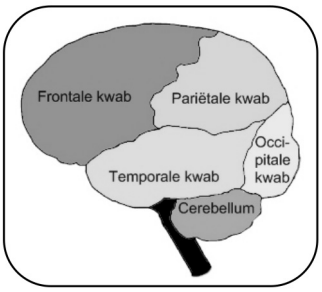
In totaal heb je 17 korte taakjes gedaan. Samen meten deze taakjes je prestatie op verschillende cognitieve domeinen. Hieronder kun je lezen wat deze domeinen betekenen. Je prestatie op deze domeinen is te zien in een grafiek.

Je scores zijn weergegeven in percentages, zo kan je zien hoe jij scoort in vergelijking met de andere jongeren die aan dit onderzoek hebben meegedaan.
Heb je bijvoorbeeld een score van 60% op een bepaald domein, dan betekent dit dat 60% van de andere jongeren lager of even hoog scoort als jij, en 40% hoger scoort. In de grafiek staat de kleur groen voor een gemiddelde score (25-75%), paars voor een benedengemiddelde score en blauw voor een bovengemiddelde score.
De scores zijn zogeheten *efficiëntie* scores, deze zijn gebaseerd op je totaal score en je reactiesnelheid.

Neurocognitief onderzoek is altijd een momentopname van je vaardigheden. Factoren als vermoeidheid, een rustige omgeving, goede verlichting en motivatie zijn belangrijk voor de prestatie op een test. Een score, zoals wordt bepaald in dit onderzoek, kan in werkelijkheid best wat hoger of lager liggen. Om de bovenstaande redenen moeten je prestaties dus met enige voorzichtigheid worden geïnterpreteerd.

Ik hoop je hiermee voldoende geïnformeerd te hebben. Mochten er nog vragen zijn dan kun je natuurlijk altijd contact met mij opnemen.

## Neurocognitieve domeinen




**Aandacht:** In de test hebben we gemeten hoe goed je de aandacht over langere tijd vol kan houden terwijl je cijfers en letters moest herkennen. Hiervoor gebruik je frontale gebieden van het brein (zie plaatje).

**Geheugen (woorden, gezichten, ruimtelijke figuren)**
Bij het onderzoek moest je na het zien van de woorden, gezichten en 3D-figuren direct, en een tijdje later weer, aangeven welke je herkende. Hoe goed je geheugen voor woorden, gezichten en figuren is kan van elkaar verschillen. Temporale gebieden van het brein zijn betrokken bij het geheugen.

**Executieve functies (werkgeheugen, abstractie en mentale flexibiliteit)**
Het werkgeheugen is een soort van tijdelijke opslagplaats (bijvoorbeeld een telefoonnummer of hoofdrekenen). Executieve functies zijn er om gedachten en acties in je hoofd te organiseren: hierbij helpt het frontale deel van het brein ons.

**Sensomotorische verwerkingssnelheid**
In deze taak moest je met de muis op groene blokjes klikken die steeds kleiner werden en zich verplaatsten. Deze taak reflecteert hoe snel je brein informatie van je ogen verwerkt en hier een reactie met de hand op aanstuurt. De sensorische en motorische cortex liggen in het frontale deel van het brein.

**Verbaal redeneren**
We hebben getest in hoeverre je in staat bent het verband tussen begrippen te zien. Tijdens het doen van deze taak heb je parietale delen van het brein nodig.

**Ruimtelijk inzicht**
Ruimtelijk inzicht is het begrijpen hoe een object kan veranderen als het draait in de ruimte (bijvoorbeeld bij kaartlezen). Bij de test moest je een lijn laten draaien totdat deze parallel liep aan de andere lijn. Ook bij deze taak worden parietale delen van het brein gebruikt.

**Emotie identificatie**
Het verwerken en begrijpen van informatie uit je sociale omgeving is een belangrijke vaardigheid. Wij hebben dit gemeten door je emoties en leeftijden te laten herkennen (boos, blij) en onderscheiden (welk gezicht is bozer of ouder?). Hierbij worden temporale hersengebieden gebruikt.

<<voornaam>> <<achternaam>>
<<straat>> <<huisnummer>>
<<postcode>> <<plaats>>

| DATUM | ONS KENMERK | UW BRIEF VAN | UW KENMERK |
|---|---|---|---|
| | NTR/brainscale | | |
| **E-MAIL** | **TELEFOON** | **FAX** | **BIJLAGE(N)** |
| s.c.swagerman@vu.nl | 020 598 5131 / 598 8827 | 020 598 8832 | 1) Toestemmingsformulieren<br>2) Verzamelmateriaal + instructie<br>3) Vragenlijsten<br>4) Retourenvelop |

Beste <<voornaam>>,

Wat leuk dat je weer mee wilt doen aan het onderzoek naar de ontwikkeling van brein en cognitie bij jongeren! Hierbij sturen wij de bevestiging van de afspraak die we telefonisch hebben gemaakt voor de testdag op het UMC Utrecht. De cognitieve taken en hersenscans zullen plaatsvinden op:
**Datum:** …… **Tijd:** …… (Het bezoek zal ongeveer 5 uur duren)

Wanneer je je meldt bij de centrale balie bij de ingang van het UMC Utrecht, word je daar vervolgens door ons opgehaald.

Bij deze brief is het volgende gevoegd:
1. Persoonlijk toestemmingsformulier (2x). Dit formulier wordt gebruikt om je deelname aan de studie te bevestigen. Lees het door en neem het mee naar de afspraak.
2. Instructiefolder urine- & speeksel verzameling
3. Verzamelbuizen urine (2x) en speeksel (2x) met retourenvelop
4. Instructie monduitstrijkje
5. Buizen monduitstrijkje
6. Vragenlijst MRI-onderzoek
7. Vragenlijst voor tweelingen vanaf 16 jaar en hun broers en zussen
8. Retourenvelop VU

Voordat je naar het UMC komt willen we graag dat je weer ochtendurine en speeksel verzamelt en een monduitstrijkje maakt. In de informatiefolder urine- & speeksel verzameling staat uitgelegd hoe dat moet. Lees de folder goed door. Dit keer mag je de buizen per post opsturen (stop ze in de kleine bubbelenvelop en verstuur in de grote retourenvelop).
Medicatie kan de testen beïnvloeden. Als je medicijnen gebruikt, wil je deze dan meenemen naar de VU?

Checklist voor het bezoek aan het UMC Utrecht:
- ✓ persoonlijk toestemmingsformulier (2x)
- ✓ toestemmingsformulier voor ouders / verzorgers (2x)
- ✓ routebeschrijving
- ✓ vragenlijst MRI-onderzoek en (doosjes) medicijnen
- ✓ als je ze niet met de post meestuurt: de urine- en speeksel buizen met de vragenlijst
- ✓ als je ze niet met de post meestuurt: de monduitstrijkjes
- ✓ vragenlijst voor tweelingen vanaf 16 jaar en hun broers en zussen
- ✓ vragenlijst voor ouders
- ✓ vragenlijst voor vader en moeder
- ✓ cd of dvd
- ✓ ter herinnering: horloges, sieraden, piercings en make-up worden verwijderd voor de MRI-scan

We verwelkomen jullie graag weer op het UMC Utrecht!
Met vriendelijke groet,
Suzanne Swagerman, MSc. (NTR, tel: 020-598 5131, e-mail: s.c.swagerman@vu.nl) en
Marinka Koenis, MSc. (UMC, tel: 088-755 3386, e-mail: m.m.g.koenis@umcutrecht.nl)

Aan de ouders / verzorgers van:
<<straat>> <<huisnummer>>
<<postcode>> <<plaats>>
<voornaam>> <<achternaam>>

| DATUM | ONS KENMERK | UW BRIEF VAN | UW KENMERK |
|---|---|---|---|
| | NTR/brainscale | | |
| **E-MAIL** | **TELEFOON** | **FAX** | **BIJLAGE(N)** |
| s.c.swagerman@vu.nl | 020 598 5131 / 598 8827 | 020 598 8832 | 1) Toestemmingsformulieren ouders<br>2) Vragenlijsten<br>3) Routebeschrijving<br>4) Retourenvelop |

Beste familie,

Hartelijk bedankt voor jullie deelname aan ons onderzoek naar de ontwikkeling van brein en cognitie bij jongeren, we zijn erg blij dat jullie opnieuw mee willen doen! Hierbij sturen wij de bevestiging van de afspraak die we telefonisch hebben gemaakt voor de testdag op het UMC Utrecht. De cognitieve taken en hersenscans zullen plaatsvinden op:

**Datum:** …… **Tijd:** …… (Het bezoek zal ongeveer 5 uur duren)

Wanneer jullie je melden bij de centrale balie bij de ingang van het UMC Utrecht, worden jullie daar vervolgens door ons opgehaald.

Bij deze brief is het volgende gevoegd:

1. Toestemmingsformulieren voor beide ouders / verzorgers. Dit formulier wordt gebruikt om de deelname van de kinderen aan de studie te bevestigen. Lees het door en neem het mee naar de afspraak in het UMC Utrecht of geef het ondertekend door beide ouders/verzorgers mee aan uw kinderen.
2. Routebeschrijving UMC Utrecht
3. Vragenlijst voor ouders. Wilt u deze vragenlijst invullen over ieder van uw deelnemende kinderen? U kunt deze opsturen met de retourenvelop.
4. Vragenlijst voor vader en moeder. Wilt u deze vragenlijst invullen over uzelf? U kunt deze opsturen met de retourenvelop.
5. Retourenvelop VU

Mochten jullie toch verhinderd zijn op de afgesproken datum, zou je ons dit dan zo snel mogelijk willen laten weten? Je kan ons ook bellen of mailen als je vragen hebt.

Checklist voor het bezoek aan het UMC Utrecht:

- ✓ persoonlijk toestemmingsformulier (2x)
- ✓ toestemmingsformulier voor ouders / verzorgers (2x)
- ✓ routebeschrijving
- ✓ vragenlijst MRI-onderzoek en (doosjes) medicijnen
- ✓ als je ze niet met de post meestuurt: de urine- en speeksel buizen met de vragenlijst
- ✓ als je ze niet met de post meestuurt: de monduitstrijkjes
- ✓ vragenlijst voor tweelingen vanaf 16 jaar en hun broers en zussen
- ✓ vragenlijst voor ouders
- ✓ vragenlijst voor vader en moeder
- ✓ cd of dvd
- ✓ ter herinnering: piercings, horloges, sieraden en make-up worden verwijderd voor de MRI-scan

We verwelkomen jullie graag weer op het UMC Utrecht!
Met vriendelijke groet,
Suzanne Swagerman, MSc. (NTR, tel: 020-598 5131, e-mail: s.c.swagerman@vu.nl) en
Marinka Koenis, MSc. (UMC, tel: 088-755 3386, e-mail: m.m.g.koenis@umcutrecht.nl)

**NTR/BrainSCALE**

## Onderzoek naar de ontwikkeling van brein en cognitie bij jongeren

# Verklaring van toestemming na kennisneming

- Ik bevestig dat de onderzoeker mij volledig heeft ingelicht over de aard en het doel van bovengenoemde studie en ik ben op de hoogte van de onderzoeksmethoden en procedures. Ik heb voldoende tijd gehad om over mijn deelname na te denken en ben in de gelegenheid geweest om vragen te stellen.
- Ik heb de informatie over dit onderzoek, die in de folder en brief worden gegeven, begrepen.
- Ik begrijp dat ik te allen tijde de medewerking aan dit onderzoek mag afbreken zonder opgaaf van reden en zonder dat dit ongenoegen zal geven.
- Ik ga ermee akkoord dat de gegevens uit het huidige onderzoek gekoppeld mogen worden aan gegevens die eerder van mij verzameld zijn.
- Ik heb toegestemd om deel te nemen aan de volgende onderzoeken:

| Toestemming voor: | | |
|---|---|---|
| * deelname aan het hormoononderzoek | 0 ja | 0 nee |
| * deelname aan het cognitieve onderzoek | 0 ja | 0 nee |
| * deelname aan het lichamelijk onderzoek | 0 ja | 0 nee |
| * opslag en analyse van erfelijk materiaal (DNA) | 0 ja | 0 nee |
| * deelname aan de MRI scan | 0 ja | 0 nee |

Naam: _____ Geboortedatum: _____

Handtekening: _____ Datum: _____

| Onderzoekers: | Handtekening onderzoekers: | Datum: |
|---|---|---|
| S. C. Swagerman | _____ | _____ |
| M. M. G. Koenis | _____ | _____ |

**NTR/BrainSCALE**

## Onderzoek naar de ontwikkeling van brein en cognitie bij jongeren

# Toestemmingsverklaring

### In te vullen door beide wettelijke ouders / verzorgers

**Ik ben gevraagd om toestemming te geven, zodat mijn kind meedoet aan het "Onderzoek naar de ontwikkeling van brein en cognitie bij jongeren":**

Naam proefpersoon:_____ Geboortedatum: _____

Ik geef **wel/geen*** toestemming voor deelname aan het onderzoek
Naam ouder/verzorger:

_____

Handtekening: Datum:

_____ _____

Ik geef **wel/geen*** toestemming voor deelname aan het onderzoek
Naam (eventuele) tweede ouder/verzorger:

_____

Handtekening: Datum:

_____ _____

| Onderzoekers: | Handtekening onderzoekers: | Datum: |
|---|---|---|
| S. C. Swagerman | _____ | _____ |
| M. M. G. Koenis | _____ | _____ |

**Hormonen**

Zoals je in de informatiefolder hebt kunnen lezen, is het voor dit onderzoek belangrijk te weten hoe ver je in de puberteit bent. Een van de manieren om hier achter te komen is door te kijken hoeveel hormonen je in je lichaam hebt. Hormonen zijn de oorzaak van het volwassen worden van je lichaam, hersenen en gedrag. Deze hormonen kunnen we meten in speeksel en urine. In deze folder staat uitgelegd hoe je speeksel en urine kan verzamelen.

**Wanneer?**

In de week voor het onderzoek verzamel je op twee achtereenvolgende doordeweekse dagen speeksel en urine. Doe dit direct na het opstaan: sommige hormonen worden alleen 's nachts aangemaakt. Op het bijgevoegde vragenformulier kun je de dag en het tijdstip invullen.

**Hoe verzamel je speeksel?**

Vul de juiste buis tot het streepje (ongeveer 2,5 cm) met speeksel (een makkelijke manier om dit te doen is door op een lepeltje te spugen en het dan in het buisje te gieten). Het is belangrijk om dit te doen voordat je je tanden poetst of ontbijt, zodat er geen bloed of voedselresten in het speeksel terecht komen.

**Hoe verzamel je ochtendurine?**

Je kan eerst in een schoongemaakt potje plassen voordat je het overgiet in de juiste buis. Deze buis hoeft maar voor driekwart vol, dus niet tot de rand.

**En dan?**

Draai de dop goed op de speeksel- en urinebuizen (niet te strak draaien anders barst de buis misschien) en bewaar deze rechtop in de koelkast. Neem de buisjes en het vragenformulier mee naar de afspraak in het UMC Utrecht.

**Meer informatie**

Heb je nog meer vragen over het onderzoek, neem dan contact op met een van de onderzoekers:

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020-598 5131
s.c.swagerman@vu.nl

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088-755 3386
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Kijk op www.brains4teens.nl of www.tweelingenregister.org voor meer informatie over het onderzoek, hersenen, hersenfuncties en puberteit en tweelingonderzoek.

**Hormonen**

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**Wanneer?**

In de eerste week van je menstruatie verzamel je op twee achtereenvolgende dagen speeksel en urine. Doe dit direct na het opstaan: sommige hormonen worden alleen 's nachts aangemaakt. Verderop lees je wanneer je dit precies moet doen. Op het bijgevoegde kaartje kun je de dag en het tijdstip invullen.

**Hoe verzamel je speeksel?**

Vul de juiste buis tot het streepje (ongeveer 2,5 cm) met speeksel (een makkelijke manier om dit te doen is door op een lepeltje te spugen en het dan in het buisje te gieten). Het is belangrijk om dit te doen voordat je je tanden poetst of ontbijt, zodat er geen bloed of voedselresten in het speeksel terecht komen.

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**En dan?**

Draai de dop goed op de speeksel- en urinebuizen (niet te strak draaien anders barst de buis misschien) en bewaar deze rechtop in de koelkast tot je ze opstuurt. Doe alle buisjes van jezelf bij elkaar in een bubbeltjesenvelop. Stop deze in de grote retourenvelop met het vragenformulier en stuur ze op, of neem ze mee naar het UMC als je urine en speeksel hebt verzameld in de week voor je afspraak.

Als je de buisjes met de post opstuurt, doe dat dan op maandag, dinsdag of woensdag. Zo liggen de buisjes niet te lang buiten de koelkast.

**De menstruatiecyclus**

Gedurende de menstruatiecyclus verandert de hoeveelheid hormonen in je lichaam. Gemiddeld duurt een menstruatiecyclus een maand (vier weken), maar langer en/of onregelmatig kan ook. Om te kunnen meten hoever je in de puberteit bent is het voor het onderzoek van belang dat je op een specifiek moment in de cyclus speeksel en urine verzamelt. In de week van de menstruatie zijn de verschillende hormonen het meest betrouwbaar te meten. Dit kan dus gebeuren voor, of na, je afspraak met ons in het UMC Utrecht. Vandaar dat we je de mogelijkheid geven om de buisjes en de vragenlijst per post naar ons terug te sturen.

**Als je regelmatig menstrueert:** verzamel de hormonen op de 6e en 7e dag na de eerste dag van je menstruatie.

**Als je onregelmatig menstrueert:** het is niet erg als je je volgende menstruatie moeilijk kan voorspellen: je wacht deze af en verzamelt urine en speeksel op de 6e en 7e dag na de start.

**Als je nog niet menstrueert:** dan maakt het niet uit wanneer. Doe het wel op twee achtereenvolgende dagen, bijvoorbeeld in de week voor het onderzoek.

**Als je de pil gebruikt:** verzamel de hormonen op de 6e en 7e dag van je stopweek, dus vóórdat je weer met een nieuwe pilstrip begint. Het is belangrijk dat je aangeeft dat je de pil gebruikt op het bijgevoegde formulier en dit mee terugstuurt naar ons.

**Daarna**

Daarnaast willen we je vragen ons te laten weten wanneer je hierná weer bent gaan menstrueren. Je kunt de datum naar één van ons mailen op de adressen die hieronder staan.

**Meer informatie**

Heb je nog meer vragen over het onderzoek, neem dan contact op met een van de onderzoekers:


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## 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 bepaaid 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 bepaien of een tweeling een- 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 aileen een beetje vloeisto£

## 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 een van de dunne buisjes. Doe aile 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 aile 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 bepaiingen 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 gevai zullen wij het overgebleven materiaai vernietigen.

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

**Vragenformulier** Vul deze vragen in en voeg ze toe bij de buisjes

**Wanneer heb je urine en speeksel verzameld?**

Dag 1 . . - . . - . . . . Tijdstip: . . : . .

Dag 2 . . - . . - . . . . Tijdstip: . . : . .

**Zijn er bijzonderheden met betrekking tot de urine- en speeksel verzameling?**

**Vragenformulier** Vul deze vragen in en voeg ze toe bij de buisjes

- Ben je ooit ongesteld geweest? 0 Ja
  0 Nee (vul alleen de laatste twee vragen in)
- Op welke leeftijd was je voor het eerst ongesteld? . . . jaar en . . . maanden
- Ben je regelmatig ongesteld?
  0 Erg regelmatig, de dag is voorspelbaar
  0 Redelijk regelmatig, meestal binnen drie dagen rond de verwachte dag
  0 Onregelmatig, het is onvoorspelbaar
- Hoe vaak ben je ongesteld?
  0 Vaker dan 1 keer per maand
  0 Iedere 28 dagen (1 keer per maand)
  0 Minder dan 1 keer per maand
  0 Minder dan 1 keer in de twee maanden
- Gebruik je op dit moment de pil?
  0 Nee
  0 Ja, sinds . . . . . Merknaam pil: . . .
- Wat is de voornaamste reden dat je de pil gebruikt?
  0 Anticonceptie
  0 Acne
  0 Pijnlijke menstruatie (buikpijn)
  0 Onregelmatige menstruatie
  0 Anders: ……

**Wat was eerste dag van je laatste menstruatie?** **<u>Dag 1</u>** . . - . . - . . . .

**Welke dagen heb je urine en speeksel verzameld?**
**(dag 6 en 7 van menstruatie / stopweek)**

**<u>Dag 6</u>** . . -. .-. . . . Tijdstip: . . : . . **<u>Dag 7</u>** . . -. .-. . . . Tijdstip: . . : . .

Zijn er bijzonderheden met betrekking tot de urine- en speeksel verzameling?

# Nederlandse samenvatting

## Hersenontwikkeling tijdens adolescentie

### Een longitudinale tweelingstudie naar de ontwikkeling van hersenstructuur en de relatie met hormoonspiegels en intelligentie

## ALGEMENE INTRODUCTIE

*Adolescentie* is de periode waarin een jongere zich ontwikkelt van een kind tot een volwassene. Het omvat de cognitieve en gedragsveranderingen die een kind vormen tot volwassene. De *puberteit* is de periode van seksuele rijping, de ontwikkeling secondaire geslachtskenmerken. Samen beschrijven puberteit en adolescentie een periode waarin, naast lichamelijke groei, grote veranderingen plaatsvinden in cognitie, gedrag, lichamelijke kenmerken, en het brein.

Hoewel de exacte biologische mechanismen die de puberteit laten beginnen niet bekend zijn, is de cascade van hormonale en fysieke veranderingen die plaatsvinden relatief helder en duidelijk. Over het hoe, wat en waarom van cognitieve veranderingen is echter nog veel discussie. Wat gebeurt er in hun hersenen dat maakt dat tieners zich gedragen zoals ze doen? Aangezien het brein een flinke reorganisatie ondergaat, is er de mogelijkheid dat ontwikkelingsprocessen mis gaan en dat tijdens de adolescentie een aantal ontwikkelings- en psychiatrische stoornissen hun eerste symptomen laten zien. Om te weten hoe en wanneer processen verkeerd kunnen gaan, is het van belang om de "ins en outs" te weten te komen van de gezonde ontwikkeling van de hersenen. Dat is een van de doelstellingen van de BrainSCALE studie.

De BrainSCALE studie is een langlopende studie (op dit moment drie metingen) bij tweelingfamilies naar de ontwikkeling van hormonen, gedrag, cognitie, en het brein. Dat is dus een perfecte set-up om de normatieve ontwikkeling van de adolescente hersenen te bestuderen. Onderzoek in tweelingen biedt de mogelijkheid na te gaan in hoeverre genetische factoren en omgevingsfactoren betrokken zijn bij deze veranderingen. In dit proefschrift heb ik gekeken naar de hormonale ontwikkeling en hoe die ontwikkeling gerelateerd is aan de ontwikkeling van de hersenen. Ook keek ik naar het netwerk van verbindingen tussen verschillende hersenregio's en hoe dat zich verhoudt tot cognitief functioneren, gemeten met een IQ test (intelligentie quotiënt).

## SAMENVATTING

In dit proefschrift zijn veranderingen in de hormoonspiegels, cognitie en hersenstructuur bestudeerd bij tweelingen en hun niet-tweeling broers en zussen. De procedure van het verzamelen van gegevens voor dit project en in het bijzonder voor de derde meting toen de tweelingen 17 jaar waren, is beschreven in Hoofdstuk 2. In Hoofdstuk 3 en 4 zijn de data van de tweelingen geanalyseerd (gemiddelde leeftijd 9 en 12 jaar); in Hoofdstuk 5 (twee metingen) en 6 (drie metingen) zijn data van de tweelingen en hun broers of zussen geanalyseerd (gemiddelde leeftijd van 10, 13 en 18 jaar).

### *Hormonale en lichamelijke ontwikkeling*

In Hoofdstuk 3 is gekeken naar geslachtshormonen en de relatie met secundaire geslachtskenmerken. Tussen 9 en 12 jaar namen de waarden van luteïniserend hormoon (LH), follikelstimulerend hormoon (FSH), estradiol en testosteron toe met een factor 2 tot 9 in zowel jongens als meisjes. In jongens en meisjes waren hormoonspiegels op 9-jarige leeftijd gerelateerd aan hormoonspiegels en secundaire geslachtskenmerken op leeftijd 12. Variatie in hormoonspiegels en secundaire geslachtskenmerken werd beïnvloed door genen en er was een genetische correlatie tussen hormoonspiegels op leeftijd 9 en secundaire geslachtsken-

merken op leeftijd 12. In meisjes beïnvloedde een gemeenschappelijke omgevingsfactor de relatie tussen estradiol en borstontwikkeling op leeftijd 12.

De schattingen van de erfelijkheid van hormoonspiegels waren hoger voor jongens dan voor meisjes. Een ander verschil tussen jongens en meisjes was dat LH op leeftijd 9 een voorspeller was voor secundaire geslachtskenmerken in meisjes op leeftijd 12, terwijl FSH op leeftijd 9 meer een voorspeller was in jongens. Beide bevindingen laten zien dat tijdens de vroege puberteit hormoonspiegels zich anders gedragen in jongens en meisjes.

***Hormonen & het brein***

Vervolgens werd de relatie tussen hormonale ontwikkeling en hersenontwikkeling onderzocht. In Hoofdstuk 4 bleek dat bij meisjes veranderingen in FSH spiegels tussen 9 en 12 jaar positief waren gerelateerd aan de veranderingen in grijze stof dichtheid [lokale concentratie van grijze stof (de neuronale cellen)]. Op 12 jaar was estradiol negatief gerelateerd aan grijze stof dichtheid. In jongens werden geen significante associaties tussen hormoonspiegels en grijze stof dichtheid gevonden. De relaties tussen hormoonspiegels en grijze stof dichtheid of verandering van de grijze stof dichtheid werden gedreven door omgevingsfactoren.

Dat er bij jongens geen relatie werd gevonden illustreert de verschillende stadia van ontwikkeling van jongen en meisjes: in jongens loopt zowel lichamelijke ontwikkeling als breinontwikkeling achter op die van meisjes. Hoewel de lichamelijke en brein ontwikkeling hand in hand lijken te gaan, blijft het de vraag of FSH en estradiol direct veranderingen in grijze stof dichtheid veroorzaken of dat de relatie indirect is via een onderliggende derde bron die zowel puberteitsontwikkeling van de hormoonspiegels als de ontwikkeling van de hersenen teweegbrengt.

***Het brein als een netwerk***

In Hoofdstuk 5 en 6 werd onderzocht hoe het witte stof netwerk zich ontwikkelt tijdens de (vroege) adolescentie en hoe dat netwerk gerelateerd is aan IQ. Dit werd gedaan door middel van graaf theorie. Voordat ik de resultaten zal beschrijven, zal ik eerst uitleg geven over graaf theorie, netwerken en connecties. Het brein kan worden gezien als een netwerk bestaande uit regio's (90 corticale en subcorticale regio's in deze thesis) die met elkaar verbonden zijn (door middel van witte stof banen in deze thesis). In dit proefschrift heb ik gekeken naar twee eigenschappen van het witte stof netwerk: globale en lokale efficiënte. Lokale efficiëntie geeft weer hoe goed informatie tussen directe buren uitgewisseld kan worden en kan worden geïnterpreteerd als lokale verwerkings- of communicatiecapaciteit. Globale efficiëntie geeft weer hoe goed informatie over het gehele netwerk uitgewisseld kan worden, oftewel de communicatie capaciteit van het hele netwerk.

De connecties tussen de verschillende regio's werden gebaseerd op structurele verbindingen tussen de gebieden. De verbindingen werden 'gewogen' met informatie over hoe 'goed' die verbinding was. Dit werd gedaan met FA (fractionele anisotropie) en streamline count. FA is een maat van witte stof (de verbindingen tussen de hersencellen) integriteit. Streamline-count is het aantal connecties dat kan worden geconstrueerd tussen twee regio's.

***Ontwikkeling van het witte stof netwerk***

Hoofdstuk 5 beschrijft een grote toename in FA-gewogen lokale en globale netwerk effici-

entie tussen 10 en 13 jaar. De sterkste toenames in lokale efficiënte werden gezien in het achterste deel van het brein. De ontwikkeling van streamline-count gewogen netwerk efficiëntie werd ook onderzocht. Tijdens het interval van 3 jaar, vonden er zowel toenames (frontale en occipitale gebieden) als afnames (subcorticale, temporale en pariëtale regio's) plaats in de lokale efficiëntie. Dit resulteerde in een netto-afname van streamline-count gewogen globale efficiëntie.

Zowel de FA als streamline-count gewogen lokale en globale efficiëntie werden voor een groot deel beïnvloed door genen, en een stabiele genetische factor beïnvloedde globale en lokale efficiëntie van het netwerk op beide metingen. Factoren die de veranderingen in globale of lokale FA netwerk efficiëntie beïnvloedden, konden niet worden ontrafeld. In verscheidene regio's waren de veranderingen in streamline-count gewogen netwerk efficiëntie toe te schrijven aan genetische factoren.

Hoofdstuk 6 beschrijft 3 longitudinale metingen en laat zien dat tijdens de puberteit de ontwikkeling van FA-gewogen netwerk efficiëntie beschreven kan worden als een derdegraads vergelijking over leeftijd. De ontwikkeling werd gekarakteriseerd door een toename van lokale en globale efficiëntie tussen 10 en 13 jaar, gevolgd door een daling tot ongeveer leeftijd 20, waarna efficiëntie weer toenam. Lokale en globale FA-gewogen netwerk efficiëntie werden mede beïnvloed door genetische factoren op alle 3 metingen (leeftijd 10, 13 en 18).

***Het witte stof netwerk & IQ***

In Hoofdstuk 5 en 6 werd ook gekeken naar de relatie tussen IQ en het witte stof netwerk, en netwerk ontwikkeling. Hoofdstuk 5 toonde aan dat veranderingen in netwerk efficiëntie waren gerelateerd aan veranderingen in IQ. Tussen 10 en 13 jaar nam de efficiëntie van het FA-gewogen netwerk toe, met de grootste stijging in het achterste deel van de hersenen. Naast de (ontwikkelings-) veranderingen in witte stof netwerk efficiëntie, lieten de tieners ook veranderingen in IQ zien: één op de zes deelnemers had een daling of stijging van meer dan één standaarddeviatie, dat wil zeggen meer dan 15 IQ punten over een periode van 3 jaar. Interessant is dat de deelnemers met de meest uitgesproken toename in IQ ook een grotere toename in globale en lokale FA-gewogen efficiëntie hadden. Deze positieve correlatie tussen verandering in IQ en verandering in lokale efficiëntie was aanwezig in frontale en temporale regio's. Deelnemers met een afname in IQ over het 3-jarig interval lieten in deze gebieden een kleine daling zien of bleven stabiel in hun netwerk efficiëntie; deelnemers met een toename in IQ hadden een toename in lokale efficiëntie in deze regio's.

Veranderingen in lokale streamline-count gewogen efficiëntie waren negatief gerelateerd aan veranderingen in IQ. Daarnaast werd er op 13 jarige leeftijd een correlatie gevonden tussen FA-gewogen netwerk efficiëntie en IQ, maar niet tussen streamline-count gewogen netwerk efficiëntie en IQ. Samen met de verschillende ontwikkelingspatronen van streamline-count en FA-gewogen netwerken, laten de verschillende bevindingen zien dat tijdens de adolescentie het streamline-count gewogen netwerk een ander aspect van het witte stof netwerk weerspiegelt dan het FA-gewogen netwerk.

Uit Hoofdstuk 6 blijkt dat de ontwikkeling van het adolescente hersennetwerk gerelateerd is aan IQ. Op groepsniveau daalde netwerk efficiëntie tussen 13 en 18 jaar. Echter, in deelnemers met een hoog IQ bleef netwerk efficiëntie stabiel in deze periode. Dit werd

gezien op zowel globaal als lokaal niveau. De correlatie tussen netwerk efficiëntie en IQ lijkt een onderdeel te zijn van de adolescente ontwikkeling: terwijl er geen correlatie tussen lokale of globale netwerk efficiëntie en IQ was op 10 jaar en slechts een geringe correlatie in enkele gebieden op de leeftijd 13, is er een FDR-gecorrigeerde significante correlatie tussen lokale efficiëntie en IQ te zien over het gehele brein op de leeftijd van 18 jaar.

De correlatie tussen IQ en lokale FA-gewogen efficiëntie werd voor een groot deel bepaald door genetische factoren die zowel FA-gewogen efficiëntie als IQ beïnvloedden. Deze genetische correlatie was zelfs groter op leeftijd 13 dan leeftijd 18. Unieke omgevingsfactoren hadden een negatieve invloed op het verband tussen netwerk efficiëntie en IQ, waardoor de geobserveerde correlaties klein waren. Deze omgevingsfactor was niet aanwezig op 10 of 18 jaar.

**SLOTOPMERKINGEN**

De doelstellingen van de studies in dit proefschrift waren om inzicht te krijgen in op het eerste gezicht zeer verschillende onderdelen (hormonen, cognitie, hersenstructuur) van de normatieve ontwikkeling tijdens de adolescente. Veranderingen in hormoonspiegels, cognitie en hersenstructuur komen niet alleen samen voor tijdens de adolescentie, maar worden ook deels beïnvloed door dezelfde genetische en omgevingsfactoren. Adolescent-specifieke ontwikkeling van de hersenen is gekoppeld aan de puberteit, en intellectueel functioneren is gerelateerd aan de ontwikkeling van het witte stof netwerk. Genen spelen een belangrijke rol in hormonale, lichamelijke, en hersenontwikkeling. Dit proefschrift biedt nieuwe en belangrijke informatie voor toekomstige studies en vraagt daarnaast om onderzoek naar een dieper begrip van de mechanismen die ten grondslag liggen aan de relaties die we hier hebben aangetoond. Een onvermijdelijke vraag is hoe het brein zich verder ontwikkeld na de leeftijd van 18 jaar, en wat de invloed van de (pre-) adolescentie daarop is. Een vierde meting van de deelnemers van BrainSCALE is het meest geschikt om die vraag te beantwoorden.

Dankwoord
List of publications
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**DANKWOORD**

Het werk achter en voor de schermen van wat de voorgaande pagina's hebben beschreven werd mede mogelijk en/of makkelijker gemaakt dankzij vele mensen. Ten eerste natuurlijk de deelnemers die van heinde en verre kwamen voor "de derde meting". Enthousiaste pubers die het vaak prima vonden om een dagje taakjes te doen en film te kijken in de scanner. En niet alleen omdat ze dan niet naar school hoefden, naast het feit dat we ook in de avonduren en weekenden testten, vroegen sommigen vrij van vakantie werk (waar ze meer zouden verdienen dan onze cadeau bon) omdat ze het onderzoek zo leuk vonden! En op het eind van de vermoeiende dag werd vaak met enthousiasme gevraagd wanneer de volgende meting zou zijn. Ik hoop snel.

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Martijn K (derde paranimf en Held van een zekere post-it), Sonja, Marc, Lara, Dienke, Jessica, Maria (tijdens mijn stage op de VU was het ook al gezellig samen), Anna (do you still stretch at the office?), Lucía (right back at ya! I hope to join you in a postdoc abroad), en alle collega's die ik ontmoet heb: bedankt voor alle gezelligheid, (lunch) wandelingen, en gedeeld leed en succes. Speciaal dank ook aan het Titel Comité (Martijn, Sonja, Jessica, Bart).

Naast collega's waren vriendschappen[1] ook erg waardevol in de soms hectische periode van mijn eigen kleine tweede puberteit [gekarakteriseerd door veranderingen in mijn hersennetwerk en IQ (verbeteringen doordat ik heel veel geleerd heb de afgelopen jaren én een deel van de IQ test uit mijn hoofd heb geleerd; en minder positieve effecten door twee hersen schuddingen); en toenames in hormoonspiegels (ik noem een cortisol en adrenaline)].

Jemima, jaren geleden doorstonden wij samen de puberteit, en kijk eens waar we beland zijn! Het heeft naar mijn mening een positieve impact op mijn hersenontwikkeling gehad. Ik ben blij dat we er voor elkaar kunnen zijn. Kristine, dank voor de altijd interessante en soms diepgaande gesprekken. Ik mis onze wandelingen nu je zo ver weg woont! Hedwig (Stoer!), Suzan, Hans: jullie weten waarvoor.

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Genen spelen een belangrijke rol in intelligentie en hersenstructuur (en vele andere eigenschappen). Bij deze wil ik graag mijn ouders bedanken voor mijn unieke genen-set, en voor de mogelijkheden die zij mij gaven om hier te komen. Jeannette, veel dank voor je pogingen om mijn cover-idee uit te werken, en super fijn dat je alles vertaald hebt naar Indesign!

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Ronald, dank voor al het bovenstaande en zoveel meer.[4]

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1 "I get by with a little help from my friends
I get high with a little help from my friends"
– The Beatles, *With a little help from my friends* (1967), lyrics by John Lennon and Paul McCartney

2 "You've got to get close to the flame
To see what it's made of
You've got to get close to the flame
To see what you're made of"
– Tarja Turunen, *My little Phoenix* (2007), lyrics by Ruud Houweling & Michiel van Zundert

3 "And the cat has been staring at me, all this time"
– Porcupine Tree, *How is your life today?* (2000), lyrics by Steven Wilson

4 Many songs to come mind

**LIST OF PUBLICATIONS**

***Journal articles***

Koenis MMG, Brouwer RM, Swagerman SC, van Soelen ILC, Boomsma DI, Hulshoff Pol HE. *Association between structural brain network efficiency and intelligence increases during adolescence.* In revision

Brouwer RM, Panizzon MS, Glahn DC, Hibar DP, Hua X, Jahanshad N, Abramovic L, de Zubicaray GI, Franz CE, Hansell NL, Hickie IB, Koenis MMG, Mather KA, McMahon KL, Schnack HG, Strike LT, Swagerman SC, Thalamuthu A, Wen W, Gilmore JH, Gogtay N, Kahn RS, Sachdev PS, Wright MJ, Boomsma DI, Kremen WS, Thompson PS, Hulshoff Pol HE. *Genetic influences on individual differences in longitudinal changes in global and subcortical brain volumes: results of the ENIGMA Plasticity Working Group.* Accepted for publication in Human Brain Mapping

Swagerman SC, van Bergen E, Kan KJ, Koenis MMG, Hulshoff Pol HE, Boomsma DI, de Geus EJC. *No Evidence of Causal Effects of Blood Pressure on Cognition in the Population at Large.* Twin Res Hum Genet. 2016;19(1):17-26.

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***Conference abstracts***

Koenis MMG, Brouwer RM, Swagerman SC, van den Heuvel MP, Mandl RCW, van Soelen ILC, Kahn RS, Boomsma DI, Hulshoff Pol HE. Structural network development and IQ during adolescence. *Poster presented at OHBM (2015), Honolulu, USA*

Koenis MMG, Brouwer RM, Swagerman SC, van den Heuvel MP, Mandl RCW, van Soelen ILC, Kahn RS, Boomsma DI, Hulshoff Pol HE. Structural network development and IQ during adolescence. *Poster presented at Neuroscience Day (2015), Edinburgh, Scotland*

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Koenis MMG, Brouwer RM, van den Heuvel MP, Mandl RCW, van Soelen ILC, Boomsma DI, Hulshoff Pol HE. Development of brain connections and intelligence in teenagers. *Oral presentation at Cognomics Symposium (2013), Nijmegen, The Netherlands.*

Koenis MMG, Hofstra WA, de Weerd AW, Romeijn N, Piantoni G, van der Werf YD, van Someren EJW, Stam CJ. Circadian changes in the functional brain network of patients with epilepsy. *Oral presentation at the ENP meeting (2009), Doorwerth, The Netherlands.*

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**CURRICULUM VITAE**

Marinka Koenis was born in Hoogwoud (Opmeer Municipality) on March 27, 1986. After she finished high school (Gymnasium, Werenfridus in Hoorn), she went to the VU University in Amsterdam to study biomedical sciences. After 3 years she obtained her bachelor's degree and continued studying at the VU for her master's degree in neuroscience. During her master, she presented her work on her first internship (on the 5-time serial reaction time task in mice) at the Endo-Neuro-Psycho meeting (currently known as the Dutch Neuroscience Meeting) and was intrigued by a talk about graph theory and its usage for diagnosis and prognosis of Alzheimer's disease. She set out to learn more about this upcoming method to characterize brain activity at the lab of Prof. dr. Kees Stam. During her second internship she did two projects under the guidance of Kees Stam and Prof. dr. Eus van Someren at the department of Clinical Neurophysiology of the VU University medical center, and the Netherlands Institute for Neuroscience. One was on the effect of sleep deprivation on the functional network of the brain; and another on the circadian pattern of the functional brain network of epilepsy patients. After she obtained her master's degree, she was determined to apply graph theory to functional or structural brain data in her PhD. This opportunity was given by Prof. dr. Hilleke Hulshoff Pol and Prof. dr. Dorret Boomsma and their longitudinal cohort of healthy young adolescents. As part of her PhD, Marinka collected data for the third assessment of the BrainSCALE study at the University Medical Center Utrecht. Currently, Marinka is involved as a postdoctoral researcher in the set-up and quality check of cognitive tasks, IQ, and MRI scans of YOUth, a new longitudinal study into development headed by Prof. dr. Chantal Kemner. In the fall of 2017 she will join the lab of Prof. dr. David Glahn at Yale University.