CHAPTER 6

HIGHLIGHTS AND GENERAL DISCUSSION

6.1 Highlights

The research in this thesis has brought forward several findings of interest to the field of adolescent brain development:

- The thinning of the cerebral cortex during adolescence is heritable and can be described by a brain-wide genetic factor with fluctuating influences across the ages that acts in addition to secondary localized influences from novel genetic factors at the different ages (**Chapter 2**).
- Reliability modelling of the inherently noisy BOLD functional MRI signal can reveal the "true" (i.e. unconfounded by measurement error) resting-state functional connectivity between brain regions; this is reflected by the improved strength in the associations of functional connectivity with various traits and by the increased heritability estimates for the reliable component of functional connectivity (**Chapter 3**).
- There is a subtle but wide-spread developmental pattern of resting-state functional connectivity during adolescence; this is described by mostly increased functional connectivity within brain networks and by decreased functional connectivity between brain networks, and in addition to sex-specific effects for the functional connectivity within the default mode network and within the salience network (**Chapter 4**).
- Resting-state functional connectivity in the adolescent brain is partially influenced by a mixture of stable genetic and common environmental factors; i.e. there is no clear evidence for fluctuating influence of a core genetic or environmental factor or indication for novel factors at the different ages (**Chapter 4**).
- Progressive aging of the brain in patients with schizophrenia is accelerated around the time of onset for the disorder, which typically occurs during adolescence, but does not appear to be associated with progressive aging measured with epigenetics in blood, although separately both are associated with polygenic risk for schizophrenia (**Chapter 5**).

6.2 General discussion

6.2.1 Maturation and sex effects on the adolescent brain

6.2.1.1 Development of cerebral cortical thickness in adolescence

In **Chapter 2**, we investigated the development of cortical thickness in the longitudinal BrainSCALE study of children and adolescents at 9, 12 and 17 years of age. We report a gradual decrease in thickness of the cerebral cortex between 9 and 12 years of age that is accelerated between 12 and 17 years of age, with the most prominent changes occurring in the sensorimotor regions (visual, sensor, and motor cortices) and the frontal pole (**Chapter 2**). Moreover, we

reported that the accelerated thinning of the cortex that occurs between the ages 12 and 17 years makes the frontal, parietal, and temporal lobes at the age of 17 years distinct from those at the ages of 9 and 12 years in hierarchical clustering analysis (**Chapter 2**).

The developmental pattern of cortical thinning is typically observed during adolescence (Gogtay et al. 2004; Sowell et al., 2007; Raznahan et al., 2011; Wierenga et al., 2014; Zhou et al., 2015), and was now revealed to accelerate and differentiate the cortex during late adolescence based on three assessments per individual in the BrainScale cohort (van Soelen et al, 2012; van Soelen et al, 2012). Despite the numerous studies on the development of the cerebral cortex, no clear consensus has been reached whether cortical gray matter peaks during childhood or shows a continuous decline from infancy onward (Walhovd et al., 2017; Wierenga et al., 2014; Fjell et al., 2015). Between the ages 9 and 12 years, we reported that a majority of the children in the BrainSCALE cohort showed only a slight decrease in global cortical thickness, with several local cortical regions showing no significant changes in thickness (**Chapter 2**; van Soelen et al., 2012; Brouwer et al., 2014). These findings would suggest a possible plateau or peak in local cortical thickness before adolescence. For average cortical thickness, the thinning of the cortex continues beyond adolescence, and does not reach a plateau until adulthood between 30 and 60 years of age (Schnack et al, 2015). The rapid changes in the cortex during adolescence that results in phenotypic separation of the cortex in late adolescence from that in late childhood and early adolescence makes the frontal, parietal, and temporal lobe prominent candidates for studying behavioral changes during adolescence from a neuroscience perspective.

6.2.1.2 Sexual differentiation in cortical thickness development in adolescence

In **Chapter 2**, we investigated possible sex effects on the development of cortical thickness in the BrainSCALE cohort. However, we did not find any significant sex effects on the development of global cortical thickness throughout adolescence (**Chapter 2**).

In previous research on the BrainSCALE cohort, the onset of secondary sexual characteristics of puberty have been associated with decreased frontal and parietal gray matter densities at the age of 9 years (Peper et al., 2009). Changes in the levels of follicle stimulating hormone (FSH) in girls has been associated with changes in grey matter density between the ages 9 and 12 years, and levels of estradiol have been associated with grey matter density at age 12 years (Brouwer et al., 2015). This suggests that puberty may trigger the reorganization of brain structures such as the thinning of the cortex during adolescence (Peper et al., 2011). Despite the influence of puberty and pubertal hormones on the development of the cortex and the difference in pubertal timing of about 1 to 2 years between the sexes (Fredriks et al., 2000; Koenis et al., 2013), no differences between the sexes were present for the *thickness* of the cerebral cortex (**Chapter 2**). Sex effects play a prominent role in explaining variation of

volumetric and surface area measures of the brain structures (Lenroot et al., 2007; Ruigrok et al, 2014; Herting et al., 2018), and could explain differences observed in behavior and cognition between the sexes (Gur and Gur, 2016). However, the presence of sex effects on cortical thickness is still disputed (Walhovd et al., 2017; Koolschijn and Crone, 2013; Sowell et al., 2007; Lenroot and Giedd, 2010; Raznahan et al., 2011). Cortical thickness and surface area are two largely independent measures that make up cortical gray matter volume (Lenroot et al., 2007; Pannizon et al., 2009). Sex effects in cortical thickness might be explained by interaction with the development of surface area in childhood and early adolescence as the brain still grows in volume (Wierenga et al., 2014; Schnack et al., 2015). Alternatively, sex effects may not necessarily be present on the means, but rather on the variances; with larger variation in males compared to females (Wieringa et al., 2018). However, we performed a qualitative analysis of sex effects in cortical thickness in **Chapter 2**, and we not find any perceivable differences in phenotypic between regions of the cortex that precludes the possibility of sex effects on the means or variances of cortical thickness.

6.2.1.3 Development of functional connectivity in adolescence

In **Chapter 4**, we investigated the development of *brain-wide* functional connectivity within and between major cortical resting-state networks. We reported that there are changes in functional connectivity during adolescence that are characterized by a remarkable developmental pattern of increasing functional connectivity within cortical resting-state networks and decreasing functional connectivity between networks with age (**Chapter 4**).

Although resting-state networks may already be established by early age (Turk et al., 2019; Gao et al., 2015), our results show that there are still wide-spread but subtle changes in the functional coupling strength between brain regions during adolescence. Only a few longitudinal studies have been performed on functional connectivity in typically developing adolescents, with most studies focusing on specific connections (see overview of literature in **Table 4.1** from **Chapter 4**). These studies generally report increasing functional connectivity within functional networks or decreasing functional connectivity between functional networks with age that is consistent with our results (Bernard et al., 2016; Long et al., 2017; Sherman et al., 2014; Wendelken et al., 2017; Wendelken et al., 2016). These developmental changes in brain connectivity may explain cognitive and behavioral changes during adolescence by tighter integration of functionally related brain regions and segregation of unrelated regions (Wig, 2017). For example, the better integration within the frontoparietal network we reported for the BrainSCALE cohort (**Chapter 4**) may support better cognitive performance (Jung and Haier, 2007). The segregation between the anterior cingulate cortex and insula of the salience network we reported for the BrainSCALE cohort (**Chapter 4**) could reflect segregation of bottom-up stimuli processing and top-down

cognitive control processing that might coincide with improved self-control and response inhibition during adolescence in reward-based decision making (Casey, 2013; Uddin et al., 2017; Bush et al., 2002; Stevens et al., 2011).

6.2.1.4 Sexual differentiation in functional connectivity development in adolescence

In **Chapter 4**, we investigated the effect of sex on functional connectivity within and between the major cortical resting-state networks. We reported that there is increased functional connectivity within the default mode network for girls compared to boys, and an opposite sex effect with increased functional connectivity within the salience network for boys compared to girls (**Chapter 4**).

Sex effects on functional connectivity have also been reported for adults and across the lifespan (Biswal et al., 2010; Zuo et al., 2010), and corroborate that sex differences in brain functioning are present during childhood and adolescence (Gur and Gur, 2016), with recent reports that sex difference in functional connectivity are already present during pregnancy in the fetal brains (Wheelock et al., 2019). These sex effects in functional connectivity could have potential relevance to behavioral differences in adolescent development. The increased functional connectivity within the default mode network for girls may explain their better performance at memory and emotive tasks for its role in auto-biography memory and emotion regulation (Gur et al., 2012; Raichle, 2015). Whereas the increased functional connectivity within the salience network for boys may explain their better performance at visuospatial and motor tasks for its role in overt attention/stimuli processing, integration of multimodal sensory information (Gur et al., 2012; Uddin, 2014).

6.2.1.5 Necessity of stringent control for head motion effects on functional connectivity development

In addition to developmental finding of functional connectivity in the BrainSCALE cohort reported in **Chapter 4**, we also report a sharp decline in the amount of in-scanner head motion during the acquisition of the resting-state fMRI scans between the ages 13 and 18 years (**Chapter 4**).

The issue of head motion during scan acquisition has raised concerns for developmental findings of functional connectivity (Power et al., 2012). Despite best efforts to control for the effect of head motion during preprocessing of the resting-state scans, significant residual effects of head motion on functional connectivity within and between resting-state networks was present and was subsequently removed with additional regression during statistical analysis (**Chapter 4**). Although head motion will likely remain an issue in developmental studies, our results should be robust due to rigorous control for the effects of head motion on functional connectivity in a longitudinal study design.

6.2.1.6 Necessity of longitudinal studies on functional connectivity development

Cross-sectional studies on the development of functional connectivity do not always show consensus on the direction of change and affected regions (Stevens, 2016). In contrast to longitudinal studies, cross-sectional findings are confounded by possible cohort effects (Mills and Tamnes, 2014; Crone and Elzinga, 2014; Telzer et al., 2018). This concern is particularly true for the development of functional connectivity given the subtle changes in functional connectivity we report in **Chapter 4** in the presence of large inter-individual variation typically present in functional connectivity and the sharp decline in head motion with age. With only a few longitudinal studies that focus on the development of specific connections for comparison, this emphasizes the need for more longitudinal studies in brain functioning to replicate the developmental findings for functional connectivity we report in **Chapter 4** for the BrainSCALE cohort.

6.2.2 Genetic and environmental influences on brain development

6.2.2.1 Genetic and environmental influences on cortical thickness in adolescence

In **Chapter 2**, we investigated the dynamic influences of genes and environment on the development of cerebral cortical thickness in the BrainSCALE cohort. We reported that the thinning of the cortex is driven by a core genetic factor with fluctuating influences from late childhood throughout adolescence, and novel genetic influences at the regional level that may reflect differential expression of genes marking distinct stages of cortical development in adolescence (**Chapter 2**).

Earlier genetic studies of cerebral cortex characteristics have reported evidence that cortical thickness for regions across the brain, including homotopic regions on each hemisphere, are influenced by a common genetic factor in both children and in adults (Schmitt et al., 2008; Rimol et al., 2010; Chen et al., 2013; Wen et al., 2016; Schmitt et al., 2017). The genetic correlation between some of these regions showed increased towards the second decade of life (Schmitt et al., 2017), that could be related to an increasing influence of the core genetic factor found in the BrainSCALE cohort (**Chapter 2**).

Moreover, we found that the core genetic factor influences both the cortical thickness measured at a specific age as well as the rate of change for the accelerated thinning of the cortex throughout adolescence (**Chapter 2**). Previous studies have reported genetic influences on the change rate of brain structures (Brans et al., 2008; van Soelen et al., 2012; van Soelen et al., 2013; Brouwer et al., 2014; Bootsman et al., 2015; Hedman et al., 2016; Brouwer et al., 2017), and how genes that drive these changes in brain volumes and cortex thickness are also responsible for intelligence (Brans et al., 2008; Brouwer et al., 2014), thinning of the cortex in schizophrenia patients (Hedman et al., 2016), and growth of cerebellar volume is related to stature during development (van Soelen et al., 2013). In a prior study of the BrainSCALE

cohort, novel genetic influences were reported for local regions of the cortex at age 12 years (van Soelen et al., 2012). In **Chapter 2**, we extended these findings with the third assessment of the BrainSCALE cohort at age 17 years, and we reported that the novel genetic influences expanded to *additional* regions of the cortex (**Chapter 2**). This genetic differentiation of the cortex during adolescence might be the result of continued areal specialization of the cortex that continues to develop well into early adulthood and beyond, in particular the frontal cortex (Schnack et al., 2015; Fjell et al, 2015).

It has been suggested that areal specialization of the neocortex is established during early development, and that later development is the result of more general maturational processes affecting the entire neocortex (Pletikos et al., 2014). Several studies reported a remarkably strong homogeneity in gene expression profiles among neocortical areas despite their functional specialization (Roth et al., 2006; Kang et al., 2011; Hawrylycz et al., 2015; Jaffe et al., 2015). These studies match the findings we reported from the BrainSCALE cohort of a core genetic factor that influences the cortical thickness throughout the entire brain and across childhood and adolescence (**Chapter 2**; van Soelen et al., 2012). However, there is a distinct temporal differential gene expression during adolescence that is marked by a second wave of substantial changes in gene expression at the end of adolescence (Somel et al., 2010; Colantuoni et al., 2011; Pletikos et al., 2014; Jaffe et al., 2015). This temporal differential gene expression of the cortex coincides with the novel genetic influences and the more pronounced waxing and waning influences of the core genetic factor we found in the BrainSCALE cohort during the accelerated thinning of the cortex between the ages 12 and 17 years (**Chapter 2**). The thinning of the cortex during adolescence has been linked to gene expression of genes related to synaptic function, dendrite development, myelination, and cellular composition of the neuropil (Whitaker et al., 2016; Kang et al., 2011; Jaffe et al., 2015). These studies support the idea that the biological processes underlying the apparent cortical thinning observed during development is due to pruning of neuronal synapses and dendrites accompanied by a decrease in supporting glial cells, and a parallel increase of oligodendrocytes responsible for myelination of neuronal axons (Huttenlocher, 1979; Bourgeois and Rakic, 1993; Huttenlocher and Dabholkar, 1997; Paus et al., 2008; Paus, 2010; Petanjek et al., 2011; Miller et al., 2012; Deoni et al., 2015). Together, the ongoing areal specialization of the cortex during adolescence might be the result of shifting balances in ongoing maturational processes and spurred by new genetic factors that could be related to the rapid cognitive and behavioral changes during adolescence.

6.2.2.2 Reliability modelling of functional connectivity for genetic studies

In **Chapter 3**, we investigated the application of a measurement model for improving the reliability of noisy measures such as functional connectivity. We reported that reliability modelling of functional connectivity can benefit genetic studies by detecting the genetic signal for the stable and reliable component of functional connectivity that is free from random measurement error (**Chapter 3** and **Chapter 4**).

Twin studies have reported genetic control over brain activity at rest with generally low heritability estimates (see overview of literature in **Table 4.2** from **Chapter 4**). Heritability estimates are influenced by the presence of measurement error (Posthuma et al., 2000). The reliable component of functional connectivity can be derived by controlling for measurement error using repeated measures or split-session measures in a measurement model (van Baal et al., 1998; van Beijsterveldt et al., 2001; Brandmaier et al., 2018; Cooper et al., 2019). An empirical evaluation of the utility of the measurement model revealed the ability to extract a reliable component of functional connectivity that had increased heritability (**Chapter 3**). This increase is due to standardizing genetic variances over the variance of the reliable component rather than the full phenotypic variance that contains the measurement error that would otherwise be attributed to an individual's unique environmental influences (Posthuma et al., 2000). The use of a measurement model resulted in similar increases in heritability estimates as previous study using a custom linear mixed effects model on repeated measures (Ge et al., 2017). This method has been published in a public repository on Github[3] and can be applied in other studies including in existing resting-state studies on fMRI brain measurements.

6.2.2.3 Genetic and environmental influences on functional connectivity in adolescence

In **Chapter 4** we applied the reliability modelling detailed in **Chapter 3** to the resting-state functional connectivity measures of the BrainSCALE cohort. We investigated the genetic and environmental influences on brain-wide functional connectivity within and between eight major cortical resting-state networks: the default mode, frontoparietal, dorsal attention, salience, sensorimotor, visual, language, and cerebellar networks. We reported that the reliable component of functional connectivity within and between the major resting-state networks is influenced by additive genetics and common environmental factors during adolescent brain development (**Chapter 4**).

So far, only a few studies have investigated genetic influences on networks beyond the default mode network. These studies were mostly performed in adults and did not always investigate influences from common environment that cannot be directly dismissed in a

[3] https://github.com/jalmar/openmx-models/reliability/

study of children living in the same household (Adhikari et al., 2018; Ge et al., 2017; Sudre et al., 2017; Yang et al., 2016). One study in particular was close to the age range of the BrainSCALE cohort and reported similar mixed influences of both additive genetics and common environment on function connectivity of resting-state networks during early adulthood (Yang et al., 2016). In both studies, connections from the frontoparietal, dorsal attention, and salience networks, all involved in higher order cognitive control, were influenced primarily by additive genetics (**Chapter 4**; Yang et al., 2016). Common environment plays a considerable role for most sensory networks, including the sensorimotor network, language network and cerebellum in the BrainSCALE cohort (**Chapter 4**) that has previously been reported only for the sensorimotor network (Yang et al., 2016). A notable exception is the default mode network that is partially influenced by an additive genetic factor or a common environmental factor in the BrainSCALE cohort instead of only an additive genetic factor typically reported by the other studies (**Chapter 4**; Adhikari et al., 2018; Ge et al., 2017; Sudre et al., 2017; Yang et al., 2016). The application of a measurement model and the use of an extended twin design may have contributed to the improved detection of common environmental influences. Although most likely a combination of both genetic and environmental influences play a role with possible interaction between the two that could not be modeled with the current limitation on sample sizes.

There was no strong indication for distinct genetic influences at the different ages or heritability of the *changes* in functional connectivity, suggesting mostly stable influences across adolescence (**Chapter 4**). This is in contrast to the dynamic genetic influences on the structural brain of the BrainSCALE cohort we reported in **Chapter 2** for the same ages. A longitudinal study of functional brain development during infancy reported age-dependent genetic effects on functional connectivity within resting-state networks (Gao et al., 2014). Although it is possible that genetic influences of brain function at rest may have stabilized during adolescence, additional longitudinal twin studies with larger sample sizes would be needed to verify these results.

Where twin studies can tell if a trait is influenced by genetic factors, a gene discovery study is needed to identify possible causal variants. In a genome-wide association study, the strength in functional coupling between brain regions in healthy adolescents was dependent on polymorphisms of a set of genes enriched for ion channels (Richiardi et al., 2015). The synchronous resting-state activity in the brain has been associated with gene expression levels in the cortex based on similarity in gene expression profiles for distal functionally connected brain regions (Hawrylycz et al., 2015). The oscillatory activity of the brain measured with electroencephalography (EEG) is associated with tissue-specific expression of genes that also play a role in psychiatric disorders (Smit et al., 2018) These

studies confirm that functional connectivity of the human brain is indeed partially determined by genetics.

6.2.3 Brain development and cognitive performance

6.2.3.1 Individual differences in intellect, cortical thickness, and functional connectivity

In **Chapter 4**, we investigated the association between intelligence and the coupling strength within and between the major cortical resting-state networks in the BrainSCALE cohort, including the frontoparietal network that has been implicated to support a distributed network of cognitive performance (Jung and Haier, 2007). However, no significant association between intelligence and functional connectivity was found for individual connections within or between the major cortical resting-state networks (**Chapter 4**). Moreover, preliminary investigation of the association between intelligence and cortical thickness in the BrainSCALE cohort revealed that the association previously reported to appear around 12 years of age (Brouwer et al., 2014) started to diminish again at age 17 years (not shown).

Previous research on the BrainSCALE cohort has reported an association between cognitive abilities and global brain volumes already at the age of 9 years that is partially determined by a genetic correlation (van Leeuwen et al., 2009). However, the phenotypic and genetic association between (verbal) intelligence and cortical thickness does not emerge until the age of 12 years (Brouwer et al., 2014). At the age of 17 years, the association between intelligence and cortical thickness started to disappear again, and is most likely explained by the reversal of the association between intelligence and cortical thickness (Schnack et al., 2015). This reversal of the association has implications for studying cognitive development in late adolescence and emerging adulthood. Other developmental studies have reported similar associations between intelligence and cortical development during childhood and adolescence that is both dependent on age with diminishing strength during late adolescence and show strong genetic overlap between the two measures (Shaw et al., 2006; Burgaleta et al., 2014; Schmitt et al., 2019).

Previously research on the BrainSCALE cohort has linked cognitive performance to increases in global network efficiency of the structural brain (Koenis et al., 2015; Koenis et al., 2018). However, no association between intelligence and functional connectivity was found for individual connections within or between the major cortical resting-state networks (**Chapter 4**). Other studies have typically reported on increased functional connectivity between regions that are part of the fronto-parietal network in association with intelligence in adults, but extends to other regions depending on the type of the cognitive process (Song et al., 2008; Hearne et al., 2016; Basten et al., 2015; Shearer et al., 2020). Contemporary theories on human intelligence suggest that cognitive performance

is attributed to the integration of a brain-wide regions that extend beyond the fronto-parietal network, rather than supported by individual regions or structural or functional connections (Jung and Haier, 2007; Deary et al., 2010; Barbey, 2017). Although, in contrast to global efficiency of the structural brain, evidence that global efficiency of the functional brain supports intelligence has been disputed (Kruschwitz et al., 2018). In general, there have been sparse number of reports on associations between functional connectivity and behavioral traits that has been attributed to unreliable measurements at the individual functional connections of the brain (Vaidya and Gordon, 2013; Geerligs et al., 2017; Noble et al., 2019). The application of a reliability model in the association between functional connectivity and traits may reveal the "true" association in absence of measurement error (Leigh Wang, 2010; Cooper et al., 2019).

6.2.3.2 Improving the associations between functional connectivity and traits with reliability modelling

In **Chapter 3**, we investigated the application of a measurement model for improving the reliability of noisy measures such as functional connectivity. Empirical evaluation of the utility of a measurement model in the Human Connectome Project Young Adult cohort for different sample sizes and scan durations revealed improvement in association strengths up to 1.8-fold (**Chapter 3**).

Associations between functional connectivity and behavioral measures are typically low for resting-state functional connectivity (Vaidya and Gordon, 2013; Smith et al., 2015). The sparse and conflicting reports of functional connectivity with measures such as intelligence (Kruschwitz et al., 2018), might be the result of unreliable measurements that puts an upper limit on the association (Vul et al., 2009; Leigh Wang, 2010). However, despite the improvements in association strength using a measurement model to obtain estimates of functional connectivity "free" of random measurement error, most traits remained only weakly associated for individual connections (**Chapter 3**). Functional connectivity might simply not be sensitive enough for detecting associations with behavioral traits at individual connections but instead requires a holistic approach (e.g. multivariate or connectome-based modelling) to find robust associations with behavior (Geerligs et al., 2017; Smith et al., 2015; Finn et al., 2015; Rosenberg et al., 2015). Alternatively, the highly adaptive and flexible nature of human behavior might be better supported by dynamic properties of functional connectivity (Hutchison et al., 2013; Hilger et al., 2020). The measurement model and example code are available on a public repository on Github[4] and can be applied in other studies including in existing resting-state studies on fMRI brain measurements.

[4] https://github.com/jalmar/openmx-models/reliability

6.2.4 Brain development and progressive aging in schizophrenia

6.2.4.1 Accelerated aging in the brain of schizophrenia patients

In **Chapter 5**, we reported on accelerated aging of brain structure for patients with schizophrenia that is nominally associated with polygenic risk for schizophrenia. The presence of accelerated aging in schizophrenia is consistent with other studies reporting accelerated aging of the brain in schizophrenia patients (Koutsouleris et al., 2014; Nenadić et al., 2017; Kaufmann et al., 2019; Jonsson et al., 2019). We reported that individuals with higher polygenic risk for schizophrenia displayed faster aging of the brain (**Chapter 5**) that is in line with reported overlap between common genetic variants associated with brain aging and common variants associated with schizophrenia in the population (Kaufmann et al., 2019). Typical development provides a baseline for aberrant development that is usually associated with psychiatric disorders such as schizophrenia (de Wit et al., 2016; Smieskova et al., 2010; Rapoport et al., 2012). The combination of polygenic risk for schizophrenia, longitudinal brain changes, and clinical measures could be used to better predict transition to psychosis (Perkins et al., 2019). However, discordance for the disorder in monozygotic twins suggests that despite the high heritability of the disorder and brain structures in general, the onset of the disorder might be a result of an interaction with the environment that is unique to each individual (Smith, 1970; van Os et al., 2008).

6.2.4.2 Epigenetic aging in schizophrenia patients

In **Chapter 5**, we reported on epigenetic aging in blood that was significantly accelerated in schizophrenia patients, but was not associated with accelerated aging in the brain. Previous reports have suggested no accelerated epigenetic aging or association with premature mortality for schizophrenia in blood or post-mortem brain samples (McKinney et al., 2017; Voisey et al., 2017; McKinney et al., 2018; Kowalec et al., 2019), with some exceptions (Okazaki et al., 2019; Ori et al., 2019). An absence of association between the MRI-derived brain age gap and Horvath's epigenetic age gap in blood samples has previously been reported in elderly population (Cole et al., 2018). No overlap between genetic variants identified for schizophrenia and epigenetic aging is reported in a relatively small sample despite indication for colocalization of genetic and epigenetic loci implicated in schizophrenia (Lu et al., 2018; Hannon et al., 2016). Moreover, epigenetic loci used to predict epigenetic age do not overlap with known epigenetic loci implicated in schizophrenia (Mill et al., 2008; Hannon et al., 2016). The absence of an association between MRI brain aging and epigenetic aging has been reason to suspect distinct aging processes are involved (McKinney et al., 2017; Voisey et al., 2017; McKinney et al., 2018; Cole et al., 2018). The absence of association with Levine's epigenetic age predictor, one that also takes into account extrinsic factors of typical aging, from the results of the schizophrenia cohort

affirms the conclusion that aging of the brain and epigenetic aging in blood might be two distinct processes in the etiology of schizophrenia, despite their commonality in predicting mortality (Cole et al., 2018; Marioni et al., 2015; Chen et al., 2016).

6.2.5 Methodological considerations

There are some methodological limitations that should be considered when interpreting the findings of this thesis. First, the BrainSCALE cohort was acquired on 1.5 Tesla MRI scanners that, in contrast to the 3.0 Tesla MRI scanners used in the schizophrenia cohort and the Human Connectome Project Young Adult cohort, produce images with lower signal-to-noise ratio (Frayne et al., 2003). Despite the availability of 3.0 Tesla MRI scanners at follow-up assessments, a deliberate choice was made to continue using the same 1.5 Tesla MRI scanners to minimize the impact of scanner variations on the brain measures. Second, although the BrainSCALE cohort has a decent sample size for neuroimaging, it is relatively modest for twin studies. The sample size limits the power of the genetic analyses in the ability to detect weak genetic signals, especially for noisy measurements such as resting-state functional connectivity (Boomsma et al., 2000). However, restrictions on the power of a study due to unreliable measurements can be partially lifted by the application of reliability modelling. Similarly, the progressive aging study in the schizophrenia cohort is limited in the availability of genetic and epigenetic samples. It should be noted that the cohort was not used to identify genetic or epigenetic loci related to progressive brain aging. Instead, the summary scores from large discovery samples were used to obtain the polygenic risk for schizophrenia and epigenetic aging scores used in the analyses. Third, although longitudinal statistical analyses were employed to take advantage of the repeated measurements in the BrainSCALE and the schizophrenia cohorts, the data were not processed using longitudinal pipelines. Longitudinal pipelines can help to minimize inter-scan variation that may arise during processing (Reuter et al., 2012; Hart et al., 2018). Finally, no longitudinal epigenetic samples were available in the schizophrenia cohort. Although epigenetic modifications are believed to be relatively stable over time (Talens et al., 2010; Shah et al., 2014), the timing of the epigenetic modifications can still play a crucial role in the manifestation of psychiatric disorders (Kofink et al., 2013).

6.2.6 General conclusion

Understanding typical brain development in children and adolescents is important because it provides a baseline for what is to be considered aberrant development, and helps to understand what brain structures or functioning is responsible for adolescent behavior. The inclusion of twins can determine to what extent variation in adolescent brain characteristics and behavior are determined by genetic and environmental influences. Together, these

studies can inform what makes some children thrive and others don't, and aid in the development of diagnostic tools and interventions to assist children who are struggling.

In this thesis, it has been established that the thickness of the cerebral cortex and its rate of development during adolescence is largely under genetic control. A new wave of influences from genetic origin marks the transition during adolescence. Aberrant development is associated with psychiatric disorders such as schizophrenia. Accelerated aging of the structural brain is in part genetically determined by genes implicated for schizophrenia, but does not show a dependable association with epigenetic modifications associated with aging phenotypes. Functional connectivity in the resting brain is also under genetic control, but to a lesser extent than brain structures, and we found indication of environmental influences from familial origin. This suggests that, while the development of brain structures might be preordained based on the genetic markup inherited from the parents, brain functioning might be more flexible to extrinsic influences that could be more easily targeted by interventions.

Future studies are needed not only to extend research on adolescent brain development and behavior, but also to validate and replicate existing research findings. Large population-based cohorts can help by prospectively including subjects that have yet to develop problems. Their statistical power will help to more reliably detect associations between the adolescent brain and behavior of smaller effect sizes. Combined with a longitudinal design and twin design, these studies could validate results from this thesis, and be used to extend the results by investigating gene-environment interactions and longitudinal statistical causal modelling that typically require large sample sizes.