EXPLORING NEURAL AND GENETIC SUBSTRATES

OF READING ABILITY

by

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To my mother,

Yuhua Chen.

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ABSTRACT

The primary research goal of this dissertation was to combine multimodal neuroimaging data to investigate the neural and genetic substrates of reading ability. We evaluated structural and functional neural measures for their association with genetic markers and with reading ability.

Chapter 2 investigated whether any of reading-related volumetric neural markers were candidate endophenotypes that were associated both with reading ability and with alleles of the *KIAA0319* dyslexia-susceptibility gene. We used structural Magnetic Resonance Imaging (MRI) to measure volumetric markers previously associated with reading in 68 adults. The results showed that volume of posterior corpus callosum (pCC) and right inferior frontal gyrus significantly predicted reading performance, and pCC volume was also significantly associated to a risk allele in the *KIAA0319* gene. These findings demonstrate that pCC volume is a plausible endophenotype linking the *KIAA0319* gene to reading ability.

Chapter 3 used diffusion tensor imaging (DTI) to explore the relationship between structural connectivity markers and both reading behavior and genetic risk. The results showed that reduced white matter integrity in the left temporo-parietal region was associated with poor reading performance. Additionally, we found that greater radial diffusivity, which suggests less insulation of myelin sheaths, in the mid-posterior corpus callosum (mpCC) were associated with dyslexia risk alleles of the *KIAA0319* gene. We propose that the effect of genetic risk on the volume of mpCC may be related to white matter microstructural changes in the region.

Chapter 4 used functional MRI to look for brain regions where neural activation during phonological processing was associated with reading ability. The identified region in the left supramarginal gyrus (SMG) was then used to

search for functional connectivity markers. We found that the strength of functional connectivity between bilateral SMG was significantly associated with reading ability, suggesting that this marker is an important neural underpinning of reading.

Taken together, our findings extend previous research on the neural and genetic basis of reading and literacy, suggest potential endophenotypes for dyslexia, and point to the importance of efficient connection among a reading-related network. This work helps to advance our understanding of the neural and genetic substrates of reading.

Chapter 1: An Overview of Genetic and Neural Markers of Reading Ability and Dyslexia

1.1 DYSLEXIA AND READING DISABILITIES

Developmental dyslexia (hereafter, dyslexia) is a neurologically based learning disability characterized by reading and spelling impairment that cannot be explained by intelligence, education or other factors (Lyon, Shaywitz, & Shaywitz, 2003; Schulte-Korne, 2010). The ability to read is a crucial skill in the modern industrialized world that allows the utilization of knowledge and information acquired by others. Although most children learn to master fluent reading skills, about 5-10% of school-age children in English-speaking countries are affected with dyslexia. It is the most common of the developmental disabilities and can persist into adulthood (Schulte-Korne, 2010).

Learning to read requires the ability to recognize and manipulate phonemes, the smallest units of sound in a language (Lyon, et al., 2003). This ability to identify and manipulate the sounds in words is generally referred to as phonological awareness. It is widely accepted that deficits in such phonological processes are at the core of reading impairments in many dyslexic readers, who therefore have difficulty mapping written language onto spoken words (Lyon, et al., 2003; Shaywitz et al., 1998). In addition to the core phonological deficit well documented in dyslexia and the reading literature, a large and growing body of research has provided evidence to support the involvement of an orthographic coding deficit in some impaired readers (van der Mark et al., 2009; van der Mark et al., 2011). Orthographic coding refers to the rapid recognition of the sequence of letters in words by vision and it plays an important role in the types and frequency of dyslexia's manifestations (Paulesu et al., 2001).

It has been difficult to arrive at a coherent categorization or set of diagnosis criteria for dyslexia. A traditional definition of dyslexia used the discrepancy between reading performance and that expected on the basis of the child's intellectual abilities, while more recent definitions do not mention this discrepancy, or even argue against including it (Siegel, 1988; Stanovich, 1988). But other researchers propose that there remains strong grounds for retaining the discrepancy criterion (Fawcett, Nicolson, & Maclagan, 2001). In this dissertation, we defined dyslexic readers as individuals with average to high IQ, but with self-reported reading difficulties and with poor reading performance on at least two standardized reading test batteries (see more details in chapter 2). In this way, we ruled out certain causes of reading impairments such as poor general intellectual ability.

Many researchers use the terms "dyslexia" and "reading disability" interchangeably, while others see dyslexia as different from reading difficulties resulting from other causes, such as a non-neurological deficiency with vision or hearing, or poor reading instruction (Stanovich, 1988; Warnke, 1999). For our study, we screened out individuals with self-reported problems in vision, hearing, or attention (e.g., ADHD), and therefore minimized the possibility that reading difficulties in our sample might be due to specific sensory or attentional deficits. One important thing to note is that, although we obtained a formal diagnosis of reading disability (i.e., dyslexic or not) from linguistic specialists, we examined individual differences in reading ability as a continuous measure instead of as a categorical dichotomy. Throughout this dissertation, we use the term "reading disability" to refer to the lower end in this reading ability continuum. This is also consistent with the fact that reading ability, like many other polygenic traits, is influenced by many genes each of which has a small effect. The result is a relatively normal distributions of phenotypes (Plomin, DeFries, McClearn, & McGuffin, 2008), with dyslexia comprising the tail end of the distribution of reading ability.

1.2 SIGNIFICANCE OF UNDERSTANDING READING MECHANISMS

Modern society is strongly dependent on the use of reading as a cultural, educational, and social medium. For individuals who have specific difficulties in learning to read, the reading problems almost inevitably impact their school experiences, restrict educational attainments and affect later life chances. In addition, it has long been clear that children with reading disabilities may also exhibit more frequent emotional and behavioral difficulties and disorders than those without reading problems (Maughan & Carroll, 2006). Put simply, reading disability could negatively impact other aspects of an individual's life, and therefore requires early screening and intervention to prevent its negative influences.

Reading disability is increasingly acknowledged to be a disorder with a genetic origin and a basis in the brain. Twin and family studies have estimated the heritability to be between 0.30 to 0.70 depending on the diagnosis criteria, age, and study design (Castle, Datta, & Grayan, 1999). The high prevalence estimates and high heritability estimates have led to great interest in understanding the neural and genetic mechanisms of reading disability and general reading processes, because doing so could lead to improved early screening of children who are at risk for dyslexia as well as new biologically based treatments.

1.3 DISSERTATION OVERVIEW

1.3.1 Specific aims

The goal of this dissertation is to investigate the relationship between genetic risk factors, neural markers of reading and dyslexia, and reading behavior. Towards this goal, we measured and analyzed reading-related structural and functional neural markers, reading ability, and several genetic markers in a group of participants. We had three specific aims:

Aim 1: We aimed to collect many of the most prominent neural markers of reading disability in the same group of participants and directly compare how well they predict reading performance. Neuroimaging techniques provide multimodal imaging data, including measures of brain structure, task-based functional

activation, and functional and structural connectivity among brain regions. Although they may be inherently interrelated, each of them reveals a unique aspect of brain functionality. Many previous studies focused on a single neural marker or neural markers from a single imaging modality, making it difficult to directly compare neural measures acquired across multiple modalities. This dissertation employed three different neuroimaging techniques, including anatomical magnetic resonance imaging (anatomical MRI), functional MRI (fMRI), and diffusion tensor imaging (DTI), to examine neural measures of volume, structural connectivity, and functional connectivity.

Aim 2: We aimed to investigate the association between genetic risk and neural measures. Many previous genetic studies have focused on associations between genetic variants and behavior without looking at genetic associations with neural measures. But reading disability has a strong neural basis, and individual differences in the neural substrate are very likely to lie on the pathway from genetic factors to behavior. Therefore, examining genetic-neural relationships will deepen our understanding and improve the interpretability of the genetic-behavior findings. Due to the scope of our study, we focused on a single gene (the *KIAA0319* dyslexia-susceptibility gene) to increase power. This gene was selected because it is the most consistently replicated dyslexia susceptibility gene so far, as is discussed in the section on genetic factors.

Aim 3: We also aimed to explore the relationship between different neural measures of reading. Although each neuroimaging technique has its unique contribution in revealing brain structure and function, neural markers across different modalities are likely to be interrelated. Exploring their relationship might add additional information relevant for predicting reading behavior and understanding the gene-brain-behavior pathways.

1.3.2 Dissertation organization

In the rest of this chapter, we review the literature on the neural and genetic factors associated with reading ability and dyslexia. Specifically, we give an overview of important neural and genetic markers found to be related to

reading behavior, followed by a review of behavioral genetic and imaging genetic studies in the reading literature.

In chapter 2, we focus on structural neural markers and use anatomical MRI to obtain volumetric measures of a set of the most prominent brain structures that previously have been associated with reading. We examine their power in predicting reading ability, as well as how strongly they are associated with two specific genetic polymorphisms in the *KIAA0319* gene that have been linked to reading and dyslexia.

In chapter 3, we analyze structural connectivity measures in white-matter pathways, and assess their relationship with reading performance and genetic risk. In addition, we examine the relationships among neural markers collected across different imaging modalities. In particular, given that the posterior corpus callosum was associated with both reading performance and genetic risk (in chapter 2), we examined how its connectivity properties (measured by diffusion tensor imaging) could contribute to understanding variance in its structural volume (measured by structural MRI).

In chapter 4, we employ functional MRI and three hierarchically ordered tasks, which were designed to tap subcomponents of reading, to examine task-based functional activation for phonological and orthographic processes. Furthermore, we use psychophysiological interaction (PPI) analysis to determine whether functional connectivity between brain regions varies as a function of different experimental tasks. Then we explore the power of different functional connectivity markers in predicting reading ability.

Finally, in chapter 5 (conclusion), we provide a general discussion of our findings in the context of prior research on behavioral and imaging genetic studies, and discuss the limitations of our research, as well as directions that future research could go. We conclude with a discussion of potential translational relevance of the current and future studies aimed at elucidating the neural and genetic substrates of reading.

1.4 NEURAL MARKERS ASSOCIATED WITH READING

1.4.1 Structural neural markers of reading and dyslexia

Using different neuroimaging techniques, anomalies of brain structure have been consistently observed and reported in poor readers. This section reviews a number of structural brain measures that have been found to be associated with reading ability and disability to date.

One of the earliest reported neural markers of reading disability was related to the size of the planum temporale. The planum temporale is a triangular shaped region constituting the heart of Wernicke's area in the posterior superior temporal gyrus. It is engaged in complex auditory processing and it exhibits a robust leftward hemispheric asymmetry in most normal readers (M. A. Eckert et al., 2008). This asymmetry has been associated with higher verbal ability (M.A. Eckert, Lombardino, & Leonard, 2001; Galaburda, Sherman, Rosen, Aboitiz, & Geschwind, 1985; Gauger, Lombardino, & Leonard, 1997; Leonard et al., 1996; Rumsey et al., 1997). For instance, leftward planum temporale asymmetry was associated with superior verbal IQ and phonological skills in a relatively large sample of sixth grade children (M.A. Eckert, et al., 2001). Planum temporale asymmetry has also been found to significantly correlate with asymmetry of the posterior Superior Temporal Gyrus gray matter, which was found to predict verbal ability (M. A. Eckert, et al., 2008). Early studies reported significantly more symmetry in the size of left and right planum temporale in individuals with reading disabilities compared with typical readers (M. A. Eckert, et al., 2008; Gauger, et al., 1997; Leonard, et al., 1996; Rumsey, et al., 1997), although other studies have failed to replicate the finding (Best & Demb, 1999; Leonard et al., 1993; Rumsey, et al., 1997; Schultz et al., 1994). Based on these previous studies, the asymmetry of planum temporale and of superior temporal gyrus gray matter was included here as a candidate structural marker of reading ability.

Individuals with reading disability are also characterized by reduced gray matter volume in inferior frontal gyrus, especially in the pars triangularis region in the left hemisphere (commonly known as Broca's area). Reading disability has

been consistently shown to be associated with decreased gray matter volume or surface area in bilateral inferior frontal gyrus across a number of prior studies (Brown et al., 2001; M. A. Eckert et al., 2003; Frye et al., 2010; Vinckenbosch, Robichon, & Eliez, 2005). Specifically, Eckert et al. found that left and right pars triangularis surface area was significantly smaller in dyslexic than in control children (M. A. Eckert, et al., 2003); Fryer et al. reported both decreased gray matter volume and surface area of inferior frontal gyrus in adult dyslexics (Frye, et al., 2010). Similarly, Vinckenbosch et al. found a positive correlation between reading performance and gray matter density in inferior frontal gyrus, with poor performance on rhyme judgment tasks associating with reduced gray matter density (Vinckenbosch, et al., 2005). Because inferior frontal gyrus is one of the most commonly identified structures with morphological abnormalities in dyslexic populations, the current study included both left and right inferior frontal gyrus gray matter volume as structural markers.

The corpus callosum (CC) is the major commissure between the cerebral hemispheres and is known to play a crucial role in interhemispheric communication. Previous studies have explored this structure in dyslexic brains using measures of area (Duara et al., 1991; Hynd et al., 1995) or of angles between specific landmarks (Robichon, Bouchard, Demonet, & Habib, 2000; Robichon & Habib, 1998). The most notable differences between dyslexic and non-dyslexic population have been found in posterior corpus callosum. However, the reported differences have been inconsistent across studies. For example, von Plessen et al. found shorter corpus callosum shape in the posterior midbody / isthmus region in dyslexics (von Plessen et al., 2002) and Castro-Caldas et al. reported a thinner posterior midbody section in an illiterate group compared to a literate group (Castro-Caldas et al., 1999). Research by Fine et al. further confirmed those findings with better readers showing a larger mid-sagittal area at the midbody of the corpus callosum (Fine, Semrud-Clikeman, Keith, Stapleton, & Hynd, 2007). However, other studies have found enlarged posterior corpus callosum in dyslexic participants. For instance, Duara et al. found that the splenium was larger in dyslexic subjects than non-dyslexic subjects (Duara, et al.,

1991) and this group difference pattern was also found in other studies (Casanova et al., 2010; Hasan et al., 2012; Robichon & Habib, 1998; Rumsey et al., 1996). In light of these many studies, we included a measure of posterior corpus callosum among our structural measures.

Gray matter volume in the left occipitotemporal cortex has also been associated with dyslexia (Kronbichler et al., 2008; Silani et al., 2005). This region, including the fusiform gyrus and posterior part of the inferior and middle temporal gyrus, contains the so called Visual Word Form Area [VWFA Cohen et al, 2000, 2002] and has been shown to be activated less in dyslexic readers compared with normal readers (McCandliss & Noble, 2003; Pugh et al., 2000; Shaywitz & Shaywitz, 2005). Silani et al. (2005) reported less gray matter density in a left posterior middle temporal region in English, French, and Italian dyslexic readers. Another morphometry study of occipitotemporal cortex found decreased gray matter volume in the left and right fusiform gyrus of dyslexic readers (Kronbichler et al., 2008), although some other studies did not reveal occipitotemporal abnormalities (Brown et al., 2001; Eckert et al., 2005). Given the important role of these areas in visual word processing and the corresponding morphological findings, gray matter volume in left fusiform gyrus and left posterior middle temporal gyrus were also included as structural markers in our study.

The cerebellum also exhibits abnormalities in dyslexic populations. Eckert et al. (2003) reported significantly smaller right anterior lobes of the cerebellum in dyslexic brains. Similar findings were reported by Kronbichler et al. where decreased gray matter volume in the dyslexic group was found in bilateral anterior cerebellum (Kronbichler et al., 2008). The volume of right cerebellum was also reported to be the best biomarker of dyslexia by brain classification techniques (Pernet, Poline, Demonet, & Rousselet, 2009). Based on these findings, we included a measure of cerebellum volume as one of our structural markers.

There are a number of other structural anomalies identified in dyslexic readers, such as altered cortical volume in the left temporal cortex (Brown, et al.,

2001; Silani et al., 2005; Steinbrink et al., 2008; Vinckenbosch, et al., 2005) and medial occipital cortex (in lingual gyrus) (Kronbichler, et al., 2008; Silani, et al., 2005). We included them as volumetric structural markers of reading ability in chapter 2.

1.4.2 Structural connectivity markers of reading and dyslexia

It is now widely accepted that successful reading requires the collaboration of distant cortical regions. Skilled reading depends on proficient processing of each reading-related brain region, and also requires efficient signal transmission within the white matter pathways that connect those regions. Therefore, it has been hypothesized that properties of axonal connections between cortical regions might systematically account for individual differences in cognitive abilities such as language and reading (Golestani, Paus, & Zatorre, 2002). In fact, reading difficulties have been associated with abnormalities in functional and structural connectivity in multiple studies (Horwitz, Rumsey, & Donohue, 1998; Paulesu et al., 1996; Pugh, Mencl, Jenner, et al., 2000).

There has been growing evidence of a significant association between reading skills and the microstructural properties of white matter pathways important to reading and language. Klingberg et al. were the first to report correlations between reading ability and white matter microstructural properties in a left temporo-parietal region in 17 adults, with better reading performance associated with increased mean diffusion anisotropy within the region (Klingberg et al., 2000). This finding was later replicated both in typical readers (Beaulieu et al., 2005; Deutsch et al., 2005) and in poor readers (Niogi & McCandliss, 2006; Odegard, Farris, Ring, McColl, & Black, 2009; Steinbrink, et al., 2008). In addition to the left temporo-parietal region, reading skills were also found to be associated with fractional anisotropy in several other brain regions, such as in bilateral frontotemporal and left frontal white matter (Steinbrink, et al., 2008). It was also recently found that the microstructural properties of the arcuate fasciculus were correlated with children's phonological awareness, which is a crucial component of skilled reading (Yeatman et al., 2011). Diffusion measurements of white matter

pathways also suggest that the left hemisphere inferior longitudinal fasciculus carries signals important for reading (Yeatman, Rauschecker, & Wandell, 2012). We included these measures of structural connectivity in our study.

1.4.3 Functional task-based activation and connectivity markers of reading and dyslexia

Studies using functional neuroimaging techniques, including task-based functional activation and functional connectivity patterns, have shed additional light on the neural architecture of reading and reading disability. A number of functional neuroimaging studies have reported associations between reading ability and task-related activation in reading-related brain regions. For example, many studies have found that individuals with reading disability exhibit reduced brain activation in the posterior temporal region and the occipito-temporal region (Brambati et al., 2006; Kronbichler et al., 2006; Shaywitz et al., 2002). A few studies have also reported that dyslexic participants activate the left inferior frontal cortex more than controls during reading (Brunswick, McCrory, Price, Frith, & Frith, 1999; Kronbichler et al., 2006; Shaywitz, et al., 1998) but these findings have not been always replicated (Maisog, Einbinder, Flowers, Turkeltaub, & Eden, 2008).

Fluent reading relies not only on the adequate activation of individual reading-related cortical regions, but also on efficient communication between these processing regions (Vandermosten, Boets, Wouters, & Ghesquiere, 2012). This hypothesis has been supported by a number of neuroimaging studies showing that reading involves a widespread network of cortical regions, predominantly in the left hemisphere, and that poor readers exhibit decreased functional connectivity among those regions. For example, Pugh et al. found that functional connectivity between the angular gyrus and four occipital and temporal regions of interest was significantly disrupted in dyslexic readers, but only during conditions that required phonological assembly (non-word rhyme and semantic category condition) (Pugh, Mencl, Shaywitz, et al., 2000). Another study also reported a significant disruption of functional connectivity between VWFA and left

inferior frontal and left inferior parietal language areas in children with dyslexia (van der Mark, et al., 2011).

Based on these prior studies, we also included task-related functional activation markers and functional connectivity markers in the current research, using hierarchically structured tasks that were designed to tap into different components of reading processes.

1.5 GENETICS OF READING AND DYSLEXIA

Dyslexia does not just occur randomly in the population. Family and twin studies have demonstrated that dyslexia has a strong genetic component. An early family study showed that an individual's risk of being affected increases when other family members are affected (Hallgren, 1950). Twin studies have long been employed to estimate the contribution of the environmental and genetic components in the etiology of dyslexia (Olson, Wise, Conners, Rack, & Fulker, 1989). Typical twin studies recruit a large set of monozygotic and same sex dizygotic twins; then researchers compare the concordance rate of dyslexia between the two groups of twins. A higher concordance rate in monozygotic twins compared to dizygotic twins would suggest a genetic influence on dyslexia; this has actually been reported consistently across many studies. Twin studies also enable an estimate of the heritability of dyslexia, which is the proportion of phenotypic variation attributable to genetic variation. The estimate has been put between 0.30 and 0.70 depending on the diagnosis criteria, age, and study design (Castle, et al., 1999).

The evidence from family studies and twin studies showing the high heritability of dyslexia has led to great interest in searching genomic regions/loci carrying quantitative trait loci for dyslexia susceptibility. Throughout the genome, a number of loci have been identified that are likely to harbor candidate dyslexia susceptibility genes. Linkage studies and association studies have helped to narrow down several genetic loci on autosomal chromosomes in Caucasian samples, for example, *DYX1C1* on chromosome 15, *KIAA0319* and *DCDC2* on chromosome 6, *ROBO1* on chromosome 3, and *MRPL19*/*C2ORF3* on

chromosome 2. Most of these susceptibility genes have been found to be associated with dyslexia status, but also with a wide range of components involved in reading in the general population (Paracchini et al., 2008; Scerri et al., 2011).

To date, the basic approach to identifying susceptibility genes has been to look for associations between observable phenotypes (reading-related behavior) and underlying genotypes. The next section briefly summarizes the behavioral genetics studies in humans that have led to the identification of these most important candidate genes.

1.5.1 *DYX1* on chromosome 15

One of the earliest reported linkages to dyslexia susceptibility was a locus on chromosome 15, from 15q15.1 to 15q21.3, which was supported by at least five independent dyslexia linkage studies (Scerri & Schulte-Korne, 2009). In addition, the finding of a translocation at 15q21-22 that co-segregated with reading problems in members of a Finnish family (Nopola-Hemmi et al., 2000) further supported this locus, *DYX1*. The breakpoints of the translocations disrupt a gene, now known as dyslexia susceptibility 1 candidate 1 (*DYX1C1*). Studies using independent samples reported the association of dyslexia risk with minor alleles[1] of two single nucleotide polymorphisms (SNPs) in *DYX1C1* (Taipale et al., 2003). Efforts to replicate these findings have produced mixed results: A number of independent studies have tested the two specific SNPs and numerous other SNPs within *DYX1C1* for association with dyslexia susceptibility, and some of them lend support to associations of this locus with reading in different samples, including Chinese sample (Zhang et al., 2012), while others did not report the associations.

1.5.2 *DYX3* on chromosome 2

Fagerheim et al. (1999) performed a genome wide search for linkage in a large Norwegian family in which dyslexia is inherited as a dominant trait. They

[1] Allele: one of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome

found a region (*DYX3*) in 2p15-p16 on chromosome 2 that co-segregated with dyslexia. Two genes in this region (*MRPL19* and *C2ORF3)* have been studied, but replication efforts have produced mixed results (Anthoni et al., 2007; Petryshen, Kaplan, Hughes, Tzenova, & Field, 2002).

1.5.3 *DYX5* on chromosome 3

A locus on Chromosome 3, *DYX5*, has been associated with dyslexia in one large four-generation family by a genome-wide scan, with *DYX5* associated with deficits in three essential components involved in reading, including phonological awareness, rapid naming, and verbal short term memory (Nopola-Hemmi et al., 2001). A later study reported association of the *DYX5* locus with speech-sound disorder in the majority of a cohort of 77 small families (Stein et al., 2004). *ROBO1* in this locus is considered a compelling candidate gene of dyslexia that is well known to play roles in axonal targeting and also in cell migration (Hannula-Jouppi et al., 2005). Hannula-Jouppi and colleagues found in a large family pedigree with 21 dyslexics that the expression of *ROBO1* from a specific haplotype[2] of the gene was absent or attenuated in affected individuals. Together with other findings of the study, it suggests that a slight disturbance in neuronal axon crossing across the midline between brain hemispheres, dendrite guidance, or other functions of *ROBO1* might contribute to a specific reading disability in humans. Therefore, *ROBO1* may influence reading behavior as a result of its effects on axonal connections.

1.5.4 *DYX2* on chromosome 6

A locus on chromosome 6 known as *DYX2* is the most consistently replicated locus that confers risk for dyslexia (Harold et al., 2006). *DYX2* is located at 6p22.3-p21.3 and spans over 15 Mb; It has been linked with both global and component reading disability phenotypes in numerous studies (Cardon et al., 1994; Cope et al., 2005; Deffenbacher et al., 2004; Francks et al.,

[2] Haplotype: a combination of alleles at adjacent loci on a chromosome that are inherited together

2004; Harold, et al., 2006; Kaplan et al., 2002; Luciano et al., 2007; Meng et al., 2005; Schumacher et al., 2006).

Studies have identified two peaks of genetic association within *DYX2* that include two candidate genes, *DCDC2* and *KIAA0319* (Paracchini et al., 2006). *DCDC2* is a gene located 500-kilobase from *KIAA0319*; the deletion and compound short tandem repeat (STR) in intron 2 of *DCDC2* has shown a significant association with multiple reading traits in a sample of 153 American nuclear families (Meng, et al., 2005).

A peak of association with reading disability was reported at a marker in the 5' untranslated region of *KIAA0319* (Kaplan, et al., 2002). A later study by Francks found a peak of association in a 77-kilobase region including the four exons of *KIAA0319* using families from the U.K. and from the U.S. (Francks, et al., 2004). The risk haplotype on *KIAA0319* was later shown to be related to selective decrease of expression of *KIAA0319* but not other genes (e.g., *DCDC2*) in the locus (Harold, et al., 2006). This gene appears to play a role in neuronal migration during brain development, and the relevant studies are reviewed in detail in section 1.4.5. Meng et al. also showed that *KIAA0319* was strongly expressed in the adult human brain, specifically in the superior parietal cortex, primary visual cortex, and occipital cortex, areas thought to be important in reading (Meng et al., 2005). Furthermore, Dennis *et al.* pinpointed the minor allele of rs9461045, a SNP on the *KIAA0319* gene, as showing the strongest association with dyslexia in their sample and most importantly, to causatively reduce the expression level of *KIAA0319* gene in both neuronal and non-neuronal cell lines which could plausibly lead to improper development of brain structures involved in reading (Dennis et al., 2009). Therefore, SNP rs9461045 is hypothesized to be functionally relevant in the development of reading disability.

The minor allele of another SNP (rs2143340) in the region of this gene has been found to predict a reading deficit in at least three independent samples of white European descendants. It was related to impairments in irregular word reading, orthographic coding choice, and single word reading in a sample of

subjects in Oxford (Francks, et al., 2004), with deficits in single word reading, single word spelling, and phonological awareness in a U.S. sample (Francks, et al., 2004), and with worse non-word reading, spelling, and overall reading in a non-impaired British population (Paracchini, et al., 2008). Unlike SNP rs9461045, SNP rs2143340 is not assumed to have a functional role itself, but is simply an effective marker of a three-SNP risk haplotype on chromosome 6p22: It is hypothesized to be in strong linkage disequilibrium[3] with other functional genetic variants that influence expression of the *KIAA0319* gene (Paracchini, et al., 2008).

Based on the previous work, we focused on SNP rs9461045 and rs2143340, both of which are associated with the *KIAA0319* gene, as genetic markers in the present study. We hypothesized that the risk alleles of these SNPs would be associated with one or more of the neural markers which in turn would be associated with reading ability.

1.5.5 Brief summary of behavioral genetic studies

Despite the important advances made in searching for susceptibility genes, we are still a very long way from understanding the genetic basis of dyslexia. As is the case for most of complex disorders such as schizophrenia (Harrison & Weinberger, 2005), the genetic risk factors identified so far together account for only a small amount of the phenotypic variation in reading ability, in contrast to its high heritability estimates in twin studies. For example, the reading-related performance of subjects with the most studied risk haplotype on chromosome 6 (Francks, et al., 2004; Paracchini, et al., 2006) is only about 0.3 standard deviations below that of subjects without the risk haplotype (Francks, et al., 2004). In addition, the minor allele of SNP rs2143340, which effectively tags this three-SNP risk haplotype and that is strongly associated with reading ability, only increases risk in people of European ancestry, and has a frequency of only 23-28%

[3] Linkage disequilibrium: the occurrence of some combinations of alleles or genetic markers in a population more often or less often than would be expected from a random formation of haplotypes from alleles based on their frequencies

even in the most severely affected dyslexics. Clearly, identifying the genes that underlie the heritability of dyslexia is a formidable challenge.

Furthermore, the genetic factors are likely to have extensive interactions with other environmental and epigenetic factors. There is also a growing consensus that dyslexia is a heterogeneous disorder with different symptoms, causes, and genetic risk factors (Castle, et al., 1999; Fisher & DeFries, 2002; Pennington, 2006). It is not surprising then that the candidate genes identified so far account for only a small portion of phenotypic variance and leave a large amount of the risk unexplained, as the same in other common, complex traits such as diabetes, heart disease, and psychiatric disorders (Harrison & Weinberger, 2005).

To understand the mechanisms of how dyslexia runs in a family and to facilitate the search for other susceptibility genes, efforts have been devoted to investigate the underlying cellular mechanisms of how the identified susceptibility genes cause dyslexia-related traits.

1.5.6 Neurobiology of dyslexia susceptibility genes

It was proposed early that at the cellular level, subtle disturbance in neuronal migration and cortex organization might play a role in reading disability (Galaburda, 1993). This hypothesis was strengthened after three of the most prominent dyslexia susceptibility genes (*KIAA0319*, *DCDC2*, and *DYX1C1*) were proven to affect neuronal migration (Gabel, Gibson, Gruen, & LoTurco, 2010). Progress has been made in human and animal work towards understanding the processes by which dyslexia susceptibility genes might influence the brain at the cellular level.

Paracchini and colleagues discovered that RNA interference (RNAi) knockdown of *KIAA0319* interrupted typical neuronal migration patterns in the developing cerebral neocortex of mouse and human fetuses 4 days after transfection[4], with disrupted neurons migrating orthogonally to the radial glia

[4] Transfection: the process of deliberately introducing nucleic acids into cells

scaffold that they typically migrate along towards their targets in cerebral cortex (Paracchini, et al., 2006). It also caused a marked change in the cellular morphology of migrating neurons, which suggested that *KIAA0319* might be required for appropriate adhesion between migrating neurons and radial glial fibers. The *KIAA0319* protein also serves with the *ROBO1* protein as transmembrane adhesion molecules and as receptors that guide axons to appropriate targets (Galaburda et al., 2006).

DCDC2 has also been hypothesized to play a role in neuronal migration based on findings from another study using RNAi knockdown (Meng, et al., 2005). This study found that when transfected with control plasmids, cells at the surface of ventricles progressed significantly further away from the ventricle surface towards the pial surface than did the cells transfected with a vector targeted against *DCDC2*. A more recent study reported both scattered heterotopia[5] within the white matter and over-migration of neurons to ectopic positions in neocortex as a result of *DCDC2* knockdown (Burbridge et al., 2008). *DYX1C1* has also been demonstrated to affect neuronal migration(Wang et al., 2006). Furthermore, it was recently found that the *DYX1C1* gene affects the expression of other genes involved in neuronal migration and nervous system development (Tammimies et al., 2013).

In summary, disrupted neuronal migration is considered to be a cellular neurobiological antecedent to reading disability. The findings from studies using animal models help advance our understanding of the link between the functions of the candidate genes and the neuroanatomic anomalies in individuals with reading disability.

1.6 COMBINING IMAGING AND GENETICS

As was made clear in the review, reading disability has been increasingly acknowledged to be a disorder of genetic origin with a basis in the brain. Using different neuroimaging techniques, a number of neural signatures have been

[5] Heterotopia: the presence of gray matter within the cerebral white matter or ventricles.

associated with reading ability and disability. On the other hand, twin studies have established a strong genetic basis for reading disability, and behavioral genetics studies have further identified a number of candidate genes for dyslexia susceptibility. Furthermore, animal models of the susceptibility genes suggest that they play an important role in neuronal migration and other neural phenotypes (e.g., axon guidance, dendrite morphology, etc.). Based on these prior studies, recent have begun combining genetics and imaging to understand the impacts of dyslexia susceptibility genes on reading-related brain morphology and function. The approach of integrating neuroimaging and genetics to assess the impact of genetic variation on the brain is termed *imaging genetics*. Imaging genetics can complement behavioral genetics by identifying the biological effects of genetic risk factors at the level of integrated neural systems (Hariri, Drabant, & Weinberger, 2006). Therefore this approach holds potential for further elucidating the effects of genes on the normal and atypical development of cognitive functions such as reading. There is an increasing number of studies adopting the imaging genetics approach to learn more about literacy and reading ability, and the rest of this section presents a brief review of these efforts.

In imaging genetics studies on reading and dyslexia, *KIAA0319* and *DCDC2* are the two susceptibility genes that have been studied the most extensively. It was recently reported that embryonic knockdown of the *KIAA0319* gene led to a significant reduction in the mid-sagittal area of the corpus callosum in male rats (Szalkowski et al., 2013). Darki et al. found that polymorphisms in several susceptibility genes (including *KIAA0319*, *DYX1C1*, and *DCDC2*) were significantly associated with differences in white matter volume in the left temporo-parietal region (Darki, Peyrard-Janvid, Matsson, Kere, & Klingberg, 2012). A polymorphism of *DCDC2* was also shown to be associated with variation in reading/language and symbol-decoding related brain regions, especially in the left hemisphere in healthy individuals (Meda et al., 2008). A genetic variant of *MRPL19/C2ORF3* locus on chromosome 2 was recently reported to be associated with white matter volume of the posterior part of the corpus callosum and cingulum (Scerri et al., 2012).

This linkage between genetic variation and brain changes is reflected not only at the level of brain structure, but also at the level of brain function. Pinel et al. reported that another risk variant of *KIAA0319*/*TTRAP*/*THEM2* is associated with less left-hemisphere asymmetry in functional activation of the superior temporal sulcus in healthy subjects (Pinel et al., 2012). They also found a variant of the *FOXP2* gene to be associated with variation of activation in the left frontal cortex. Additionally, a recent study using Electroencephalography (EEG) reported significant attenuation of the mismatch negativity component (MMN) in both dyslexic children and their unaffected siblings in comparison to controls, suggesting alterations of neurophysiological process in children with dyslexia and those with a genetic risk for dyslexia (Neuhoff et al., 2012).

1.7 BRIEF SUMMARY

The primary research objective of this dissertation was to combine multimodal neuroimaging data to investigate the genetics, behavioral phenotypes, and neural substrates of reading ability. As noted earlier, the dissertation consisted primarily of three specific aims. The first aim was to further our understanding of the neural substrates of reading and dyslexia. To pursue this goal, I analyzed imaging data including structural volume (Chapter II), structural connectivity (Chapter III), and functional connectivity (Chapter IV), and examined the link between those neural measures and behavioral reading performance. The second aim of the dissertation was to examine the relationship between genetic risk for dyslexia and the prominent neural markers. Therefore for the prominent neural measures in Chapter II-IV, we analyzed their association with genetic risk of a specific dyslexia susceptibility gene, and further discussed how genetic risk might influence brain structures and functionality. The third aim was to explore the relationship between neural markers acquired from different imaging modalities in Chapter II, III, and IV.

Previous studies enable us to understand how different candidate gene alleles correspond to specific morphological or functional alterations of the brain. But one limitation of this prior work is that different neural markers are examined

separately and have not been compared within the same subject sample, making it hard to see the relative power of different neural measures in predicting reading performance. Furthermore, very few studies have explored the possibility that some of those neural markers may serve as endophenotypes, linking dyslexia-susceptibility genes to reading phenotypes.

In this dissertation we evaluated a variety of well-known cognitive traits, brain structural measures (including volumetric and structural connectivity measures), and brain functional measures (including task-related activity and functional connectivity measures) in terms of their association with prominent genetic markers and with reading assessment results. Given the small sample, we focused on only two specific relatively well-replicated SNPs (*i.e.*, rs9461045 and rs2143340 in the *KIAA0319* gene) that were previously found associated with reading ability. In particular, we assessed how the previously reported neural measures were associated with both reading ability and with risk status of the two reading-associated variants of the *KIAA0319* gene.

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Chapter 2: Exploring Structural Brain Markers of Reading

Abstract

A number of neural markers have been discovered using neuroimaging that are associated with reading ability. In this chapter, we investigated whether any of these neural markers were candidate endophenotypes that were associated both with reading ability and with genetic risk related to the *KIAA0319* gene. We recruited 397 participants, genotyped variants in the *KIAA0319* dyslexia-susceptibility gene, and assessed reading ability and IQ in 145 of them. We then selected 68 adults with a range of reading scores and used structural Magnetic Resonance Imaging to measure neural markers previously associated with reading disability. The size of posterior corpus callosum and right inferior frontal gyrus significantly predicted reading performance. Posterior corpus callosum volume was also significantly related to a risk variant in the *KIAA0319* gene. These results demonstrate that posterior corpus callosum volume is a plausible endophenotype linking *KIAA0319* to reading ability and suggest the promise of using neural markers to identify other susceptibility genes.

2.1 INTRODUCTION

A number of structural brain measures have been found to be associated with reading ability and disability. These neural markers include asymmetry of the planum temporale (Eckert et al., 2008; Gauger, Lombardino, & Leonard, 1997; Leonard et al., 1996; Rumsey et al., 1997), cortical volume in the left temporal cortex (Brown et al., 2001; Silani et al., 2005; Steinbrink et al., 2008; Vinckenbosch, Robichon, & Eliez, 2005), the inferior frontal gyrus (Brown, et al., 2001; Eckert et al., 2003; Frye et al., 2010; Vinckenbosch, et al., 2005), medial occipital cortex (in lingual gyrus) (Kronbichler et al., 2008; Silani, et al., 2005), and cerebellum (Eckert, et al., 2003; Kronbichler, et al., 2008; Pernet, Poline,

Demonet, & Rousselet, 2009), as well as the size or shape of posterior corpus callosum (Casanova et al., 2010; Castro-Caldas et al., 1999; Duara et al., 1991; Fine, Semrud-Clikeman, Keith, Stapleton, & Hynd, 2007; Hasan et al., 2012; Hynd et al., 1995; Robichon & Habib, 1998; Rumsey et al., 1996; von Plessen et al., 2002).

In this study, we investigated whether any of these structural brain measures are candidate endophenotypes linking the *KIAA0319* gene to reading ability. Endophenotypes are measureable, usually quantitative, intermediate traits that are associated with the observable phenotype, but are more directly linked to the underlying genotype (Gottesman & Gould, 2003). Endophenotypes could be biochemical markers, neurophysiologic results, neuroanatomical measures, neuroimaging findings, or behavioral phenomena — the critical point is that an endophenotype should be intermediate between the genotype and phenotype.

We measured reading ability, assessed genetic risk related to the *KIAA0319* dyslexia-susceptibility gene, and used structural MRI to measure neural markers previously associated with reading disability. We then investigated whether any of the structural brain measures were significantly associated with both reading ability and with genetic risk.

Identifying genetic correlates of complex behavioral phenotypes often requires testing thousands of subjects, both because genetic-behavior associations are usually weak and because of the large number of genetic variants that are often considered in such analyses. Studying neural measures, like those analyzed in the current study, can help, assuming that some of those neural measures are more strongly associated with the underlying genotype than is behavior (i.e., they serve as endophenotypes, (Gottesman & Gould, 2003)). Nevertheless, looking for genetic correlates of neural measures would still likely require significantly more subjects than we recruited.

We therefore focused on the much more modest goal of testing whether two single nucleotide polymorphisms (SNPs) were associated with neural measures of reading. The first SNP (rs9461045) has been found to be associated with dyslexia and to lead to reduced expression of the *KIAA0319*

gene. It is therefore hypothesized to be functionally relevant in the development of reading disability (M. Y. Dennis et al., 2009). The second SNP (rs2143340) is actually in the neighboring *TTRAP* gene and is not assumed to be functionally relevant, but simply to be in strong linkage disequilibrium with genetic variants that influence *KIAA0319* gene expression. This SNP has been found to be significantly associated with reading deficits in three independent samples of European descent (Francks et al., 2004; Paracchini et al., 2008). To further increase power, we enriched the sample by recruiting a disproportionate number of subjects who were risk carriers for neuroimaging, so that we had similar numbers in the risk carrier and non-risk carrier groups. This kind of approach has worked in a number of other studies with sample sizes comparable to or smaller than ours (Bueller et al., 2006; Hariri et al., 2002; Hariri & Weinberger, 2003).

2.2 METHODS

2.2.1 Subjects

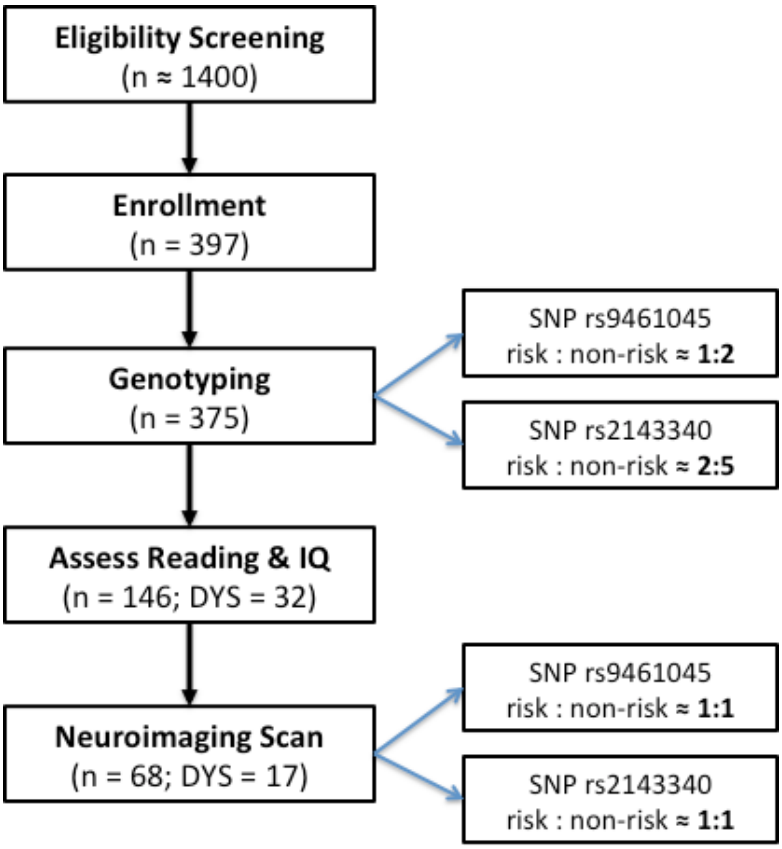

Figure 2.1 Flow diagram showing the number of participants in each of the five phases, eligibility screening, enrollment, genotyping, reading & IQ assessment, and Imaging.

397 native English-speaking, right-handed subjects with no reported psychiatric diagnoses participated. Figure 2.1 presents a depiction of the flow of participants through eligibility screening, enrollment, genotyping, behavioral assessment, and neuroimaging. Subjects ranged in age from 16–39 years (mean 23.0, standard deviation 4.8). There were 237 females and 160 males. The average education level was 15.0 years (standard deviation 2.2). All participants and, where appropriate, parents, gave written informed consent in accordance with a protocol reviewed and approved by the Health Sciences and Behavioral Sciences Institutional Review Boards (IRB-HSBS) at the University of Michigan. 68 subjects were selected for neuroimaging based on risk genotype carrier status, IQ, and reading score. Among the 68 participants (ages 16-39 with mean of 22.4), 33 were females, and 17 were diagnosed as developmental dyslexics (ages 16 to 31 years with mean of 22.2) with no reported comorbid mental disorders (e.g., ADHD). Linguistic specialists made the diagnosis based on self-reported reading difficulty, composite and subtest scores of two standardized reading test batteries, and a discrepancy between IQ and reading performance (see Table 2.2 for reading and IQ assessment results of dyslexic and control groups). Note, however, that the current study examined reading ability as a continuous measure rather than treating dyslexics and controls as two discrete groups.

2.2.2 Polymorphism genotyping

All participants gave a saliva sample for genetic analysis. The saliva samples were collected and DNA extracted using an Oragene Saliva kit (DNA Genotek, Kanata, Ontario, Canada). We genotyped the DNA samples from all our participants for two SNPs associated with the *KIAA0319* gene (rs9461045 and rs2143340) that have previously been associated with reading ability. Genotypes were determined using Taqman assays according to manufacturer specifications (Life technologies, previous ABI).

2.2.3 Reading tests

Participants were invited back for IQ and Reading Tests to assess reading ability and to inform dyslexia diagnosis. The following assessments were performed on 146 eligible participants: (a) Wechsler Abbreviated Scale of Intelligence (WASI), including subtests of vocabulary and matrix reasoning; (b) Woodcock-Johnson III (WJ-III) (Woodcock, McGrew, & Mather, 2001) Diagnostic Reading Battery which provided measures of Letter-word Identification, Reading Fluency, and Passage Comprehension. (c) Test of Word Reading Efficiency (TOWRE) (Torgesen, Wagner, & Rashotte, 1999) which included Sight Word Efficiency (SWE) and Phonemic Decoding Efficiency (PDE). Table 2.1 provides a brief description and example(s) for each reading subtest used in the current study.

Table 2.1 Brief description and example(s) of each reading subtest.

Subtest Name	Subtest Description	Example(s)
Woodcock-Johnson III (WJ-III)		
Letter-word Identification	Naming letters and reading words aloud from a list.	since, achieved, domesticated
Reading Fluency	Speed of reading sentences and answering yes/no to each.	A bird can fly. ..... Y N
Passage Comprehension	Orally supplying the missing word removed from each sentence or very brief paragraph.	"Woof," said the ____, biting the hand that fed it.
Test of Word Reading Efficiency (TOWRE)		
Sight Word	Number of words correctly read within 45 seconds.	is, up, work, jump, crowd, better, uniform…
Phonemic Decoding	Number of non-words correctly read within 45 seconds.	ip, ga, lat, baf, knap, tive, guddy, skree …

2.2.4 Structural MRI acquisition

68 subjects were selected for neuroimaging based on risk genotype carrier status, IQ, and reading ability. High-resolution anatomical images were collected for each subject using a spoiled 3D gradient-echo acquisition (SPGR) pulse sequence on the 3-Tesla MRI scanner at the University of Michigan's Functional Magnetic Resonance Imaging Laboratory. A standard head coil was used and participant movement was minimized by stabilizing the head with cushions. The field of view was 259 mm, voxel size was 1×1×1.2 mm (123 axial

slices), TR (repetition time) was 9 msec, TE (echo time) was 1.8 msec, flip angle was 15°, and the whole structural scan lasted about 6 minutes.

2.2.5 Structural MRI analysis

We used the FreeSurfer (Fischl et al., 2002) software package (http://surfer.nmr.mgh.harvard.edu) to automatically parcellate and calculate the volume of specific cortical and subcortical structures in each individual. The NIFTI format of the high-resolution anatomical image was first converted to FreeSurfer's mgz format. FreeSurfer automatically segments the volume and parcellates the surface into standardized regions of interest and provides volume measurements for multiple cortical and subcortical regions. We analyzed brain volume for the following neural structures: planum temporale (left and right), BA 45 (left and right), pars triangularis (left and right), triangularis subdivision in inferior frontal gyrus (left and right), fusiform gyrus (left), fusiform in occipitotemporal cortex (left), lingual gyrus (left), lingual gyrus in occipitotemporal cortex (left), angular gyrus (left), bilateral cerebellum-cortex, and mid-posterior and posterior corpus callosum. To measure the structural asymmetry of planum temporale, an asymmetry ratio was calculated by dividing the volume of left planum temporale by the volume of right planum temporale for each individual.

2.2.6 Factor Analysis

Freesurfer provides multiple different parcellations of the brain and so a number of different Freesurfer regions overlap (e.g., left BA 45, left pars triangularis, and left inferior frontal gyrus). We therefore performed exploratory factor analysis to combine related measures and reduce the dimensionality of the data. We first applied Barlett's test of sphericity to ensure that sufficient correlations existed among the markers and that a factor analysis was appropriate. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy for each raw neural marker was then computed, and those markers with the smallest KMO were removed until the KMO of every included marker was above 0.5 and the overall KMO was also above 0.5. Based on these criteria, the left angular gyrus volume and the asymmetry ratio of planum temporale were not correlated highly with the other markers. They were therefore excluded from the factor

analysis and were treated as separate covariates in the subsequent regressions. There were 17 neural measures included in the final factor analysis. The Kaiser-Meyer-Olkin Measure of Sampling Adequacy was 0.70 and Bartlett's test of sphericity was significant (X^2 (136) = 958.67, $p < 0.0001$), suggesting that the data from the remaining markers was appropriate for factor analysis. The factor axes were rotated using varimax rotation to simplify the solutions and to make the factors more interpretable.

2.3 RESULTS

2.3.1 Behavioral results

Table 2.2 presents the standardized scores for behavioral measures of 67 subjects (one subject's performance on reading and IQ was not assessed). The IQ scores were above average as might be expected in the Ann Arbor area. Performance on the reading and intelligence measures was significantly better in the controls (who were above average on all measures) than in the dyslexics (who were below average on almost all measures).

Table 2.2 Behavioral measures with subtests indented (standard deviations are provided in parentheses; standardized scores for WASI subtests were not available, therefore raw scores are reported).

	All Subjects (N = 67)	**Controls** (N = 50)	**Dyslexics** (N = 17)
WASI	117.9 (11.2)	120.6 (9.5)	109.8 (12.3)
WASI vocabulary	63.1 (10.1)	65.9 (8.5)	54.9 (10.3)
WASI matrix reasoning	32.7 (8.2)	33.3 (9.3)	30.9 (2.2)
WJ-III	112.6 (15.6)	118.3 (12.8)	95.9 (10.5)
Letter-word Identification	103.8 (10.1)	107.5 (7.3)	93.1 (9.7)
Reading Fluency	112.8 (17.8)	118.6 (15.4)	95.6 (12.5)
Passage Comprehension	109.1 (11.1)	111.5 (10.0)	102.3 (11.4)
TOWRE	98.7 (15.3)	105.3 (10.3)	79.4 (10.4)
Sight Word Efficiency	97.9 (13.7)	102.8 (10.4)	83.6 (12.3)
Phonemic Decoding Efficiency	100.0 (14.3)	106.0 (10.4)	82.3 (8.2)

2.3.2 Neural markers predicting reading measures

Factor analysis was performed on the structural measures to reduce the dimensionality of the data and it identified six interpretable factors (see Table 2.3),

each of which was named based on the brain structures that loaded most heavily on that factor: left inferior frontal gyrus (F1), right inferior frontal gyrus (F2), fusiform gyrus (F3), lingual gyrus (F4), cerebellum (F5), and posterior corpus callosum (F6). Two other structural markers (left angular gyrus and planum temporale hemispheric asymmetry) were not highly correlated with any other markers and were therefore excluded from the factor analysis. To examine the relationship between neural markers and reading behavior, the six factors as well as the two excluded markers were entered into a multiple regression model to assess their ability to predict two different composite reading scores (the Test of Word Reading Efficiency (TOWRE) and the Woodcock-Johnson III Diagnostic Reading Battery (WJ-III)). Both models included IQ and total intracranial volume as nuisance covariates.

Table 2.3 Factor loadings of 17 initial structural measures.

	Factor Naming					
	left IFG	**right IFG**	**Fusiform**	**Lingual**	**CRBL**	**PostCC**
lh BA45	0.788					
lh G_front_inf-Triangul	0.848					
lh parstriangularis	0.875					
lh STG_transv	0.163					
rh BA45		0.730				
rh G_front_inf-Triangul		0.832				
rh parstriangularis		0.901				
lh G_oc-temp_lat-fusifor			0.860			
lh fusiform			0.927			
lh G_temporal_middle			0.559			
lh G_temp_sup-Lateral			0.403			
lh G_oc-temp_med-Lingual				0.919		
lh lingual				0.936		
lh Cerebellum					0.919	
rh Cerebellum					0.978	
CC_Mid_Posterior						0.979
CC_Posterior						0.551

Table 2.4 presents the analysis of TOWRE score. The overall regression model was significant (R^2 = 0.40; F = 3.64; p = .001). The size of right inferior frontal gyrus and of the posterior corpus callosum were significant predictors of TOWRE score (right IFG: standardized β = .34, t = 2.90, p = .005; posterior corpus callosum: standardized β = .26, t = 2.48, p = .016). Figure 2.2 presents partial residual plots that illustrate the relationship between each neural marker and reading performance controlling for the effect of all the other factors in the model. For this figure, we computed the residuals of models in which the behavioral score was entered as the dependent variable and all the markers and nuisance covariates, except for the marker of interest, were entered as independent variables. These residuals were then plotted against the marker of

interest to illustrate the relationship between the marker of interest and reading performance while controlling for the other factors.

Table 2.4 Multiple regression model of structural markers predicting TOWRE score (the model included IQ and total intracranial volume as nuisance covariates).

	Zero-order r	Standardized β	*T*	Sig.
F1: left IFG	0.175	0.186	1.575	0.121
F2: right IFG	0.185	0.337	2.898	0.005**
F3: Fusiform	0.006	0.000	-0.002	0.999
F4: Lingual	0.016	0.150	1.132	0.263
F5: CRBL	-0.020	0.013	0.105	0.917
F6: PostCC	0.293	0.259	2.475	0.016*
PT ratio	0.051	0.042	0.395	0.694
AnG Vol	0.002	-0.075	-0.598	0.552
IQ score	0.469	0.502	4.526	< 0.001**
Intracranial	0.044	-0.179	-0.941	0.351

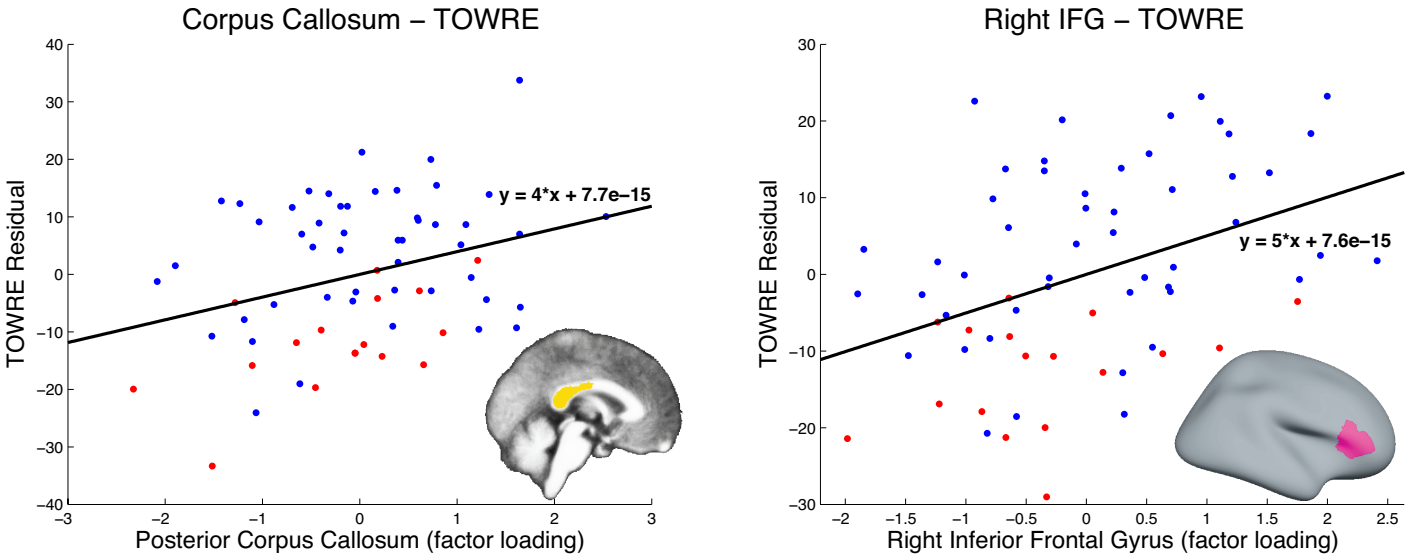

Figure 2.2 Partial residual plots of significant relationships between structural markers and TOWRE reading score. Red dots indicate participants who were diagnosed as dyslexic. Blue dots indicate non-dyslexic participants.

Table 2.5 presents the analysis of Woodcock-Johnson III (WJ-III) score. The regression model predicting WJ-III score was also significant (R^2 = 0.30; *F* = 2.33; *p* = .02). The only neural factor that significantly predicted WJ-III score was

posterior corpus callosum size (standardized β = .43, t = 2.01, p = .049). Figure 2.3 presents a partial residual plot of the relationship between posterior corpus callosum size and WJ-III score while controlling for the effect of all the other factors in the model.

Table 2.5 Multiple regression model of structural markers predicting WJ-III score (the model included IQ and total intracranial volume as nuisance covariates).

	Zero-order *r*	Standardized β	*T*	Sig.
F1: left IFG	0.147	0.128	0.987	0.328
F2: right IFG	0.070	0.165	1.298	0.200
F3: Fusiform	0.130	0.060	0.388	0.699
F4: Lingual	-0.025	0.037	0.256	0.799
F5: CRBL	0.118	0.106	0.799	0.428
F6: PostCC	0.264	0.230	2.011	0.049*
PT ratio	0.011	0.021	0.176	0.861
AnG Vol	0.074	-0.015	-0.109	0.914
IQ score	0.459	0.438	3.613	0.001**
Intracranial	0.143	-0.059	-0.286	0.776

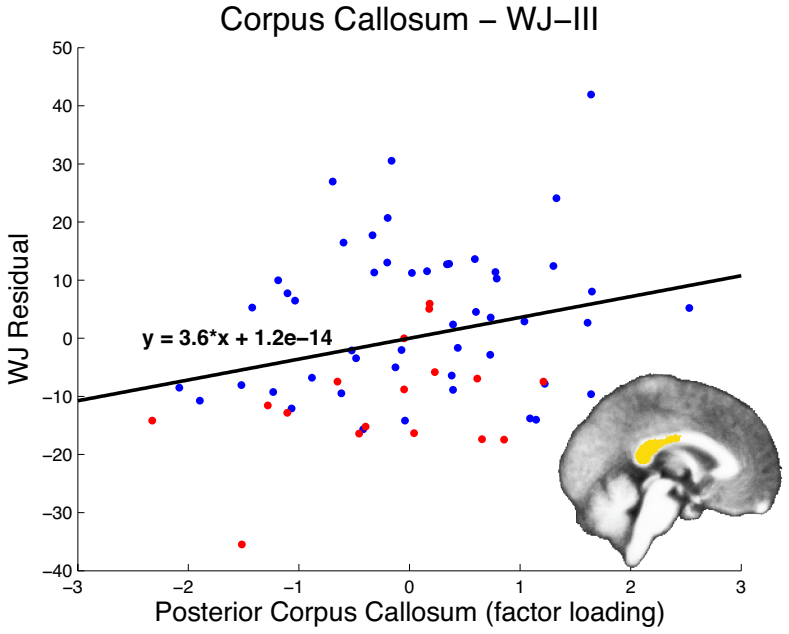

Figure 2.3 Partial residual plots of significant relationships between posterior corpus callosum and WJ-III reading score. Red dots indicate dyslexic participants and blue dots indicate non-dyslexic participants.

2.3.3 Genetic variation predicting neural markers

Next we tested whether any of the neural markers that were significantly associated with reading (posterior corpus callosum, right IFG) were also significantly associated with reading-related variants of the *KIAA0319* gene.

For each SNP, we grouped participants into risk carriers (who carried at least one risk allele: T for rs9461045; C for rs2143340) and non-risk carriers (who did not carry a risk allele). As expected from the previous known Caucasian allele frequencies, in the 375 subjects we genotyped, there were many more subjects who were not risk carriers (the ratio of non-risk carriers versus risk carriers was approximately 2:1 for rs9461045 and 5:2 for rs2143340). Hence we selected a subsample of the two groups that were matched on IQ and reading scores (see Table 2.6) to participate in the brain imaging session. We also included a disproportionately large number of dyslexics in both groups in order to balance the number of participants at the two ends of the reading scale. For the genetic-neural relationship analysis, we have genotype information from 67 participants for SNP rs9461045 and 62 participants for SNP rs2143340.

Table 2.6 Description of behavioral test scores of different genotype groups based on SNP rs9461045 and rs2143340 respectively.

	WJ-III score (mean / std)	TOWRE score (mean / std)	IQ score (mean / std)	No. of dys (dys / all)
rs9461045 (1 undetermined)				
risk group	114.9 (16.6)	100.4 (15.8)	119.1 (11.6)	7 / 36
non-risk group	110.0 (14.4)	96.8 (14.7)	116.6 (10.9)	10 / 31
total	112.9 (15.6)	99.1 (15.1)	118.2 (11.1)	17 / 67
rs2143340 (6 undetermined)				
risk group	113.5 (16.6)	97.7 (16.7)	117.9 (12.3)	6 / 30
non-risk group	111.8 (15.0)	99.5 (14.2)	117.9 (10.5)	11 / 32
total	113.0 (15.8)	99.0 (14.9)	117.9 (11.3)	17 / 62

Multiple regression analyses were applied to examine how well genetic risk predicted individual differences in each neural marker. The model also included the two reading scores, IQ and total intracranial volume as nuisance covariates. Since posterior corpus callosum volume and right IFG volume were both negatively associated with reading ability in our study, we hypothesized that risk carriers would exhibit less volume in posterior corpus callosum and right IFG.

Therefore we carried out a one-tailed test of significance for the regression analyses.

Risk status for SNP rs9461045 (N's risk vs. non-risk = 36, 31) was a significant predictor of smaller posterior corpus callosum size (standardized β = -0.22, t = -1.83, p = .035, one-tailed), after controlling for IQ, total intracranial volume, and reading ability. In contrast, genetic risk status was not significantly associated with right IFG volume (standard β = -0.09, t = -0.78, p = .44, one-tailed). The genetic risk status of rs2143340 was not a significant predictor of either the posterior corpus callosum volume (t = -1.03, p = 0.307) or the right IFG volume (t = -1.30, p = 0.198).

2.4 DISCUSSION

Previous studies have reported a number of structural brain markers associated with reading ability. Using a sample that spanned a range of reading ability from proficient readers to diagnosed dyslexics, we found that the size of posterior corpus callosum and of right inferior frontal gyrus, exhibited the strongest association with reading performance. Both were significantly associated with TOWRE score, and the size of posterior corpus callosum was also significantly associated with WJ score. The TOWRE is specifically designed to assess reading speed and efficiency whereas the subtests of WJ are more associated with accuracy, so we hypothesize that these neural markers are themselves primarily associated with reading efficiency rather than accuracy.

This hypothesis is consistent with previous work on compensatory processes in adult dyslexics (Shaywitz et al., 2003). Approximately one-fifth of individuals with dyslexia manage to compensate for their underlying learning difficulties and develop adequate reading skills by the time they reach adulthood (Lyytinen, Erskine, Aro, & Richardson, 2007). Lefly and Pennington found that compensated readers appeared very similar to non-dyslexic readers in their reading and spelling skills, but showed differences in the automaticity with which they apply these skills (Lefly & Pennington, 1991). The participants in the present study were adults and many of the poor readers among them had likely developed skills to improve reading accuracy, but their reading efficiency was still

impaired. TOWRE may therefore be a more sensitive measure of reading ability in our population, so it is not surprising that the neural markers showed a stronger association with TOWRE compared to WJ.

Consistent with prior studies, we found that the size of the right inferior frontal gyrus was a good predictor of reading ability, with reduced gray matter volume associated with worse reading performance (Brown, et al., 2001; Eckert, et al., 2003; Frye, et al., 2010; Vinckenbosch, et al., 2005). Language processing and reading are traditionally associated with the left hemisphere, but in our study, we found that the size of the right inferior frontal gyrus was strongly associated with reading performance. Consistent with this finding, recent work is demonstrating an important role of the right hemisphere in language, perhaps as a way to compensate for weaker left hemisphere function in language development. For example, many structural studies have also revealed a significant association between reading ability and the size of right inferior frontal gyrus (Eckert, et al., 2003; Frye, et al., 2010; Welcome, Chiarello, Thompson, & Sowell, 2011).

Furthermore, in a recent functional study, Hoeft and her colleagues (2011) showed that the development of reading ability in dyslexia involves greater dependence on a right-hemisphere pathway, while the development of reading ability in non-dyslexic children involves greater dependence on a left-hemisphere pathway (Hoeft et al., 2011). In their study, greater right prefrontal activity during a phonological processing task significantly predicted future long-term reading gains in individuals with dyslexia. The results pointed to the importance of right prefrontal cortex in reading improvement in dyslexics.

Our findings are consistent with this interpretation. Since all participants recruited in our study were over 16 years old, one possible explanation is that the observed association between right inferior frontal gyrus volume and reading performance in the current study is a result of individuals' enduring efforts to recruit the right-hemisphere pathway to promote reading development. For participants who were poor readers in childhood, perhaps those individuals who

exploited the right-hemisphere pathway to a greater extent improved more over time.

The corpus callosum factor predicted both TOWRE and WJ-III reading scores in the current study. The role of posterior corpus callosum in dyslexia is still controversial: Some studies (like ours) have reported a less developed corpus callosum in dyslexic readers (Castro-Caldas, et al., 1999; Fine, et al., 2007; von Plessen, et al., 2002) but others have reported increased mid-sagittal surface area of corpus callosum in dyslexics (Casanova, et al., 2010; Hasan, et al., 2012; Robichon & Habib, 1998; Rumsey, et al., 1996). An interesting study by Carreiras et al. investigated how literacy changed the brain by comparing structural brain scans from individual who learned to read as adults (late-literates) with those from carefully matched illiterates. They found that late-literates showed greater volume in the splenium of the corpus callosum (Carreiras et al., 2009). Peterson et al. also reported greater white matter intensity in the mid-body region of the corpus callosum in their literate group compared to their illiterate group (Petersson, Silva, Castro-Caldas, Ingvar, & Reis, 2007).

The posterior corpus callosum size in the current study reflects the volume of both the splenium and isthmus. The splenium of the corpus callosum mainly contains axons that connect left and right parietal and occipital cortices (Abe et al., 2004; Park et al., 2008), which include regions essential to word recognition and also low-level visual processing, such as lingual gyrus and primary visual cortices. The isthmus connects left and right language areas in the parietal and superior temporal cortices, including Wernicke's area and planum temporale that play important roles in auditory processing. The reduced size of posterior corpus callosum in poor readers therefore suggests that efficient reading depends on communication and integration of visual and phonological information between the two hemispheres, which fits well with models that hypothesize orthographic and phonological processing impairments in reading disability (Hoien & Lundberg, 2000; Manis, Seidenberg, Doi, McBride-Chang, & Petersen, 1996; Pugh et al., 2000). Although it is still unclear whether the neural abnormalities are a cause of reading difficulty or whether they are a consequence (e.g., because of less

exposure to reading instruction), a recent study found that kindergarteners who are at risk for dyslexia already showed underdevelopment of the left arcuate fasciculus, suggesting a white matter basis of risk for dyslexia before formal reading instruction (Saygin et al., 2013).

In addition to the association with behavioral reading measures, the size of posterior corpus callosum was also significantly associated with genetic risk: This brain region was significantly smaller in participants who were risk carriers for SNP rs9461045, a genetic variant considered functionally relevant for development of reading ability (M.Y. Dennis et al., 2009). This result is consistent with a recent finding in male rats that embryonic *KIAA0319* knockdown led to a significant reduction in the midsagittal area of the corpus callosum (Szalkowski et al., 2013). An association similar to our finding was also recently reported between posterior corpus callosum size and a different genetic variant associated with reading disability (the MRPL19/C2ORF3 locus on chromosome 2) (Scerri et al., 2012). Another recent study found that SNPs on several dyslexia susceptibility genes including *KIAA0319* were significantly associated with white matter volume in the left temporo-parietal region (Darki, Peyrard-Janvid, Matsson, Kere, & Klingberg, 2012).

These results suggest that one way the *KIAA0319* gene may affect reading behavior is by influencing the corpus callosum and associated interhemispheric communication. The *KIAA0319* protein is thought to play a role in neuronal migration during brain development (Paracchini et al., 2006), to help guide axons to appropriate targets (Galaburda, LoTurco, Ramus, Fitch, & Rosen, 2006), and to help the growth and differentiation of dendrites (Peschansky et al., 2010). Reduced expression of *KIAA0319* could therefore impair the coordinated changes required for axon growth in the development of the corpus callosum, thus leading to a less developed corpus callosum in those individuals carrying risk alleles. The effect might be stronger for axons in the posterior corpus callosum because this region develops earlier than other sections and it connects left and right parietal and occipital cortices where the *KIAA0319* gene has been found to be most highly expressed even after individuals reach adulthood (Meng

et al., 2005). Given that these regions play a crucial role in reading, reduced expression of the *KIAA0319* gene could therefore impact reading performance. This hypothesis is consistent with recent reviews associating dyslexia with impaired axon guidance and dendrite connectivity (Gabel, Gibson, Gruen, & LoTurco, 2010; Kere, 2011). In particular, Hannula-Jouppi and colleagues have proposed a similar hypothesis about how *ROBO1*, another susceptibility gene associated with axon guidance, might influence interhemispheric axon crossing and contribute to reading problems (Hannula-Jouppi et al., 2005).

Sample sizes in imaging studies are typically much smaller than those used in behavioral genetics studies, and hence do not lend themselves to screening thousands of variants. We tried to overcome this limitation by focusing on two genetic variants and by enriching our sample both for risk carriers and for individuals with diagnosed dyslexia, but it will be important to replicate the findings in other independent samples. On the other hand, the fact that we did obtain significant results in a relatively small sample may also reflect the power of the endophenotype approach. These results raise the exciting possibility of using neural markers to find other, as yet undiscovered, genetic factors that contribute to reading ability and of examining the neural and genetic underpinnings of different subtypes of reading disability. To the extent that the neural markers are more strongly associated with genetic risk factors than are behavioral measures, such an approach could be a significantly more powerful way to identify the genetic risks underlying reading disability.

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Chapter 3: Exploring Structural Connectivity Markers of Reading

Abstract

This chapter focused on structural connectivity measures of reading using the diffusion tensor imaging (DTI) technique, and explored whether any structural connectivity markers could serve as neural endophenotypes that link genetic risk to reading behavior. We found that white matter integrity in the left temporo-parietal region was associated with reading performance, with good readers showing greater white matter integrity in the region. Additionally, we found that increased radial diffusivity, an index of reduced myelination, in the mid-posterior corpus callosum (the isthmus) was associated with two risk alleles in the *KIAA0319* gene. Specifically, radial diffusivity of the isthmus was greater in risk carriers, suggesting less-insulating myelin sheaths in the region. We propose that the previously observed effect of genetic risk on the volume of the isthmus may be related to white matter changes in the region. In particular, a smaller isthmus in poor readers may be partially due to thinner or less integral myelin sheaths wrapping around the callosal axons in the region.

3.1 INTRODUCTION

Prior work showed that the minor allele of rs9461045 leads to reduced expression of the *KIAA0319* gene that could plausibly lead to improper development of brain structures involved in reading, providing a functional mechanism underlying the association between *KIAA0319* and dyslexia. Our findings in chapter 2 identified the volume of posterior corpus callosum as a promising endophenotype that was associated with both genetic risk and reading performance, suggesting that one way the *KIAA0319* gene may affect reading behavior is by influencing the corpus callosum and associated interhemispheric communication of language-related brain regions. These findings raise the

possibility that brain connectivity markers may be promising endophenotypes linking dyslexia-susceptibility genes and reading outcome. Therefore, the following studies aim at measuring reading-related structural and functional connectivity, and investigating whether any of those connectivity markers are associated with reading ability or genetic risk. In this chapter specifically, we examined structural connectivity measures in several reading-related white-matter regions or structures. The next chapter explores the same questions using functional connectivity measures.

3.1.1 Structural connectivity markers related to reading and dyslexia

Performing any kind of cognitive task usually requires coordination among multiple brain regions that are distributed in different parts of the brain. In the case of reading, our brain integrates signals from brain regions specialized in visual, phonological and linguistic processing in order to read text. Prior studies found that reading is performed by a network of frontal, temporo-parietal, and occipito-temporal regions, predominantly in the left hemisphere. It has been proposed that two distinct neural routes in the left hemisphere are particularly involved in skilled reading: a dorsal phonological route and a ventral orthographic route (Jobard, Crivello, & Tzourio-Mazoyer, 2003; Sandak, Mencl, Frost, & Pugh, 2004; Schlaggar & McCandliss, 2007). The phonological route is likely to be composed of the left temporo-parietal junction (including the posterior superior temporal gyrus, the angular gyrus, and the supramarginal gyrus) and areas in and around inferior frontal gyrus. This dorsal route has been associated with deciphering written words through grapheme-to-phoneme mapping. On the other hand, the orthographic route involves the left occipito-temporal sulcus near the fusiform gyrus and has been associated with fluent reading and automatic processing of visual word forms (McCandliss, Cohen, & Dehaene, 2003).

Skilled reading depends on proficient processing of each reading-related gray matter region, and also requires efficient signal transmission within the white matter pathways that connect those regions. There has been cumulating evidence of a significant association between reading skills and the microstructural properties of white matter pathways important to reading and

language. For instance, the arcuate fasciculus is considered an important language pathway that maps acoustic features of speech in Wernicke's area to articulatory motor representations in Broca's area (Hickok & Poeppel, 2004, 2007), and it was found recently that the microstructural properties of the arcuate fasciculus are correlated with children's phonological awareness, which is a crucial component of skilled reading (Yeatman et al., 2011). Diffusion measurements of white matter pathways also suggest that the left hemisphere inferior longitudinal fasciculus carries signals important for reading (Yeatman, Rauschecker, & Wandell, 2012).

In keeping with these findings, it has been hypothesized that properties of axonal connections between cortical regions might systematically account for individual differences in cognitive abilities such as language and reading (Golestani, Paus, & Zatorre, 2002). For example, researchers have found that increased myelination allows faster and more efficient processing of complex temporal acoustic signals such as speech (Golestani, et al., 2002). In fact, reading difficulties have been associated with abnormalities in functional and structural connectivity in multiple studies (Horwitz, Rumsey, & Donohue, 1998; Paulesu et al., 1996; Pugh et al., 2000).

Therefore it is plausible that certain dyslexia risk genes could alter diffusion properties of axon connections in reading-related brain regions and reduce the efficiency of signal transmission in the network, resulting in less skilled reading. To test this hypothesis, this study focused on prominent axonal connectivity markers that have been associated with reading ability and disability in prior work, and investigated their relationship with reading behavior, gross structural measures, and genetic risk variants of the *KIAA0319* gene.

3.1.2 Diffusion Tensor Imaging

In the past two decades, diffusion tensor imaging (DTI) has been used to provide specific information about the microstructural integrity and directional orientation of white matter tracts, by measuring the degree to which the diffusion of water molecules in the brain is constrained by white matter tracts. The free diffusion of water molecules at body temperature is ~3 μm per millisecond.

However, water molecules in white matter tracts are constrained by the cell membrane and myelin sheaths, which is an insulating material wrapping around nerve axons, and are more likely to diffuse along the axis of the fiber tract than in other directions. This is referred to as anisotropic diffusion, as opposed to equal (isotropic) diffusion in all directions. When diffusion takes place in an inhomogeneous medium (e.g., white matter), it can no longer be described by a scalar diffusion coefficient, because it is impeded to different degrees in different directions. Basser et al. introduced the diffusion tensor model to summarize multi-directional diffusivity measurements within a voxel, where a diffusion tensor is a 3 x 3 symmetric positive-definite matrix that describes the diffusion in all directions (Basser, Mattiello, & LeBihan, 1994). The tensor *D* is estimated by measuring the apparent diffusion coefficient in at least six independent directions, where the apparent diffusion coefficient is proportional to the velocity of the diffusing water molecules in the measured matter. The diffusion tensor model is visualized as an ellipsoidal surface that represents the mean diffusion distance of a water molecule within the voxel. The ellipsoid is characterized by its principle directions (eigenvectors) and their lengths (eigenvalues).

Several parameters are commonly derived from the ellipsoidal model (Le Behan et al., 2001). Suppose the three terms λ_1, λ_2 and λ_3 are the eigenvalues of the diffusion tensor, sorted by their magnitude in descending order. When the diffusion is anisotropic (different in different directions), the first eigenvalue (λ_1), which represents diffusivity along the principal axis of the fiber tract, can be substantially higher than the second (λ_2) and third (λ_3) eigenvalues.

Fractional anisotropy (FA) is an important scalar that is most commonly reported (Basser, 1995; Basser, et al., 1994; Basser & Pierpaoli, 1996). The FA value ranges from zero to one, with higher values related to the tendency of white matter tracts within a given voxel to be oriented in the same direction. In well-organized and well-myelinated white matter tracts, axonal bundles would be expected to be oriented in a coherent direction and restrict the diffusion of water molecules within the bundles. They should therefore show a higher FA compared to axonal bundles in less-organized white matter. FA is proportional to the

normalized standard deviation of the three eigenvalues (Basser & Pierpaoli, 1996) and is computed using the following formula (<λ> represents the average of all three eigenvalues):

$$\mathrm{FA} = \sqrt{3[(\lambda_1 - \langle\lambda\rangle)^2 + (\lambda_2 - \langle\lambda\rangle)^2 + (\lambda_3 - \langle\lambda\rangle)^2]}/ \sqrt{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}.$$

A number of DTI studies have reported associations between reading behavior and microstructural properties of white matter in different brain regions. Klingberg et al. were the first to report correlations between reading ability and FA values in a left temporo-parietal region in 17 adults, with better reading performance associated with increased FA values (Klingberg et al., 2000). This finding was later replicated in both typical readers (Beaulieu et al., 2005; Deutsch et al., 2005) and in poor readers (Niogi & McCandliss, 2006; Odegard, Farris, Ring, McColl, & Black, 2009; Steinbrink et al., 2008). But other studies have failed to find such a relationship (Andrews et al., 2010; Dougherty et al., 2007). In addition to the left temporo-parietal region, reading skills were also found to be associated with FA value in several other brain regions, such as in bilateral frontotemporal and left frontal white matter (Steinbrink, et al., 2008).

Previous work using DTI also showed that diffusivity measures in the corpus callosum were related to reading performance, but the direction was the opposite of FA-reading relationships found in other regions or tracts. For example, Frye et al. found that nine adult dyslexic readers exhibited a higher FA value in the splenium of the corpus callosum than 18 typical readers (Frye et al., 2008). A similar finding was also reported in children, with better phonological decoding skills correlated with lower FA in the left posterior corpus callosum (Odegard, et al., 2009). Dougherty et al. also reported a positive correlation between phonological awareness and radial diffusivity perpendicular to the main axis of the callosal fibers that connect temporal lobes (Dougherty, et al., 2007).

Based on this previous work, we explored the relationship between DTI measures of structural connectivity and reading behavior as well as the relationship between structural connectivity and genetic risk associated with the

KIAA0319 gene. We also investigated how the diffusivity properties relate to structural volume at the macroscopic level.

Our findings on brain structures in chapter 2 showed that poor readers and genetic risk carriers for SNP rs9461045 tended to have a smaller volume in the posterior corpus callosum. The reduced volume could be due to a number of possible changes of white matter at the microstructural level, including but not restricted to reduced thickness of myelin sheath, decreased axonal diameter, and reduced number of axons within the region (Beaulieu, 2002; Ben-Shachar, Dougherty, & Wandell, 2007), all of which might impact the efficiency of communication (bandwidth) among cortical areas. We hypothesized that the microstructural properties of the region would offer insights into the underlying neurobiological mechanism of the gross volume variation. Therefore, we also tested whether diffusivity measures of the posterior corpus callosum were associated with its structural volume.

One important point to note is that callosal tracts in the splenium (the posterior subdivision of the corpus callosum) and the isthmus (the mid-posterior subdivision) project to functionally distinct brain regions and have different microstructural properties (e.g., axon fiber density, ratio of large-diameter and thin fibers, etc.). Therefore we analyzed the diffusivity measures of these two subdivisions of the corpus callosum separately.

Of course, the fibers passing through posterior corpus callosum extend into each hemisphere. We therefore also investigated diffusivity measures in regions to which fibers in the posterior corpus callosum project. Specifically, we were interested in the forceps major, which is composed of fiber tracts in the splenium radiating into the occipital lobe, and the left tapetum, which is formed by some fibers in the posterior corpus callosum extending inferiorly along the lateral wall of the lateral ventricle into the temporal lobe.

In the current study, we used a region-of-interest (ROI)-based approach to measure diffusion properties in the left temporo-parietal region, posterior corpus callosum, and the white matter tracts extending from posterior corpus callosum into different parts of the cerebral cortex. We then examined the relationship

between the diffusivity measures in those ROIs and reading behavior. Furthermore, we investigated whether any of these measures were associated with variants of the *KIAA0319* gene.

3.2 METHODS

3.2.1 Subjects

The diffusion data described in this chapter were collected from the same set of 68 participants described in chapter 2. See section 2.2.1 in chapter 2 for a detailed description of our sample.

3.2.2 Polymorphism genotyping

See section 2.2.2 in chapter 2 for a description of the polymorphism genotyping process.

3.2.3 Diffusion Tensor Imaging acquisition

The diffusion-tensor-imaging dataset was acquired in a 7 minute 15 second scan for each subject. A total of 16 whole brain volumes were obtained for each subject, which included 15 volumes with diffusion gradient applied along 15 non-parallel directions ($b = 800$ s/mm^2) and 1 volume without diffusion weighting (T2*, $b = 0$ s/mm^2). Each volume consisted of 39 contiguous axial slices (slice thickness = 3 mm, inter-slice skip of 0.1 mm). DTI data was collected using a ramp-sampled, dual-spin-echo, single-shot, echo-planar-imaging sequence (repetition time = 8 seconds, echo time = 86.7 milliseconds, matrix = 128 × 128, field-of-view = 22cm). Also a spiral-based field map was acquired right before the DTI data acquisition.

3.2.4 DTI data analyses

The raw diffusion data were processed using the DTI tools in FSL (FMRIB [The Oxford Centre for Functional Magnetic Resonance Imaging of the Brain] Software Library, http://www.fmrib.ox.ac.uk/fsl). Eddy current correction was performed using the eddycorrect tool. Field map distortion correction was applied on all the DWI/EPI images using the field map correction tool FUGUE. Subsequently, those eddy-current- and distortion-corrected images were used to calculate diffusion-tensor elements, which were further used to calculate fractional anisotropy maps using DTIFit/FDT. DTIFit is FSL software that fits a

diffusion tensor model at each voxel of the diffusion images and generates 3D images at the same matrix size and resolution as the original diffusion images. Diffusion maps were then normalized into MNI space using the tract-based spatial statistic (TBSS) package, which is also part of FSL. After preprocessing, all participants' diffusion images were spatially normalized into MNI space using nonlinear registration.

We then carried out region-of-interest analyses on multi-subject diffusion data. As mentioned previously, the left temporo-parietal region, the posterior corpus callosum (isthmus and splenium separately) and the white matter tracts extending from it were of particular interest to us. The left temporo-parietal region is typically described as including the angular gyrus and supramarginal gyrus in the inferior parietal region together with the posterior superior temporal gyrus (Richlan, Kronbichler, & Wimmer, 2009). Since the left temporo-parietal region is a combined region that has not been specifically identified or labeled in white-matter tract atlases, we used the center MNI coordinate from Klingberg et al.'s original study (Klingberg, et al., 2000) to draw our ROI in this region. For the posterior corpus callosum, it is one of the few white matter regions that can be discretely identified by anatomical MRI. Therefore we used the segmentation outcome of the anatomical MRI data from FreeSurfer to define ROIs for each individual participant in their own native space; in this way, we avoided distortions that might be introduced in the process of normalizing individual diffusion images to a common template. In addition, in order to examine white matter tracts that extend from corpus callosum to brain regions important to reading, including forceps major and tapetum, we drew the ROIs based on the ICBM (International Consortium for Brain Mapping) atlas, a white-matter parcellation map created by hand-segmentation of a standard-space average of tensor maps obtained from 81 normal subjects (Mori et al., 2008).

After the diffusivity measures were extracted from these ROIs, we tested their associations with reading performance and genetic risk using general linear models. For the isthmus and the splenium, we also tested whether the diffusivity measure was associated with the structural volume of the region.

3.3 RESULTS

3.3.1 Diffusivity measures predicting reading performance

We first asked whether the white matter integrity in the left temporo-parietal region predicted reading performance. To answer this question, we drew an ROI in this region, using the center of the reported left temporoparietal region in Klingberg et al.'s study (center MNI coordinate [-31, -30, 16], radius = 5 mm, volume = 515 mm^3). The fractional anisotropy measure in this ROI was a significant predictor of TOWRE score (linear regression: β = 175.2, *SE* = 77.3, *t* = 2.27, *p* = 0.027) and marginally significant predictor of WJ-III score (linear regression: β = 155.9, *SE* = 80.4, *t* = 1.94; *p* = 0.057) when including IQ and total intracranial volume as nuisance covariates (Figure 3.1).

Next we tested whether the diffusivity measure in the tapetum was related to reading behavior. This region (596 mm^3) was defined using the ICBM white-matter parcellation map. We found that the fractional anisotropy in this region significantly predicted TOWRE score (linear regression: β = 106.0, *SE* = 47.9, *t* = 2.21, *p* = 0.031, Figure 3.1), and although the direction was consistent, it was not a significant predictor of WJ-III score (linear regression: β = 70.3, *SE* = 50.4, *t* = 1.39, *p* = 0.169). Total intracranial volume and IQ were also included as nuisance covariates in these linear models.

Similar analyses were performed to determine whether the FA-values in the isthmus and the splenium were significant predictors of reading performance. Neither measure was significantly associated with TOWRE score (isthmus: *t* = 0.02, *p* = 0.873; splenium: *t* = - 0.07, *p* = 0.589) or WJ-III score (isthmus: *t* = 0.02, *p* = 0.898; splenium: *t* = 0.02, *p* = 0.903).

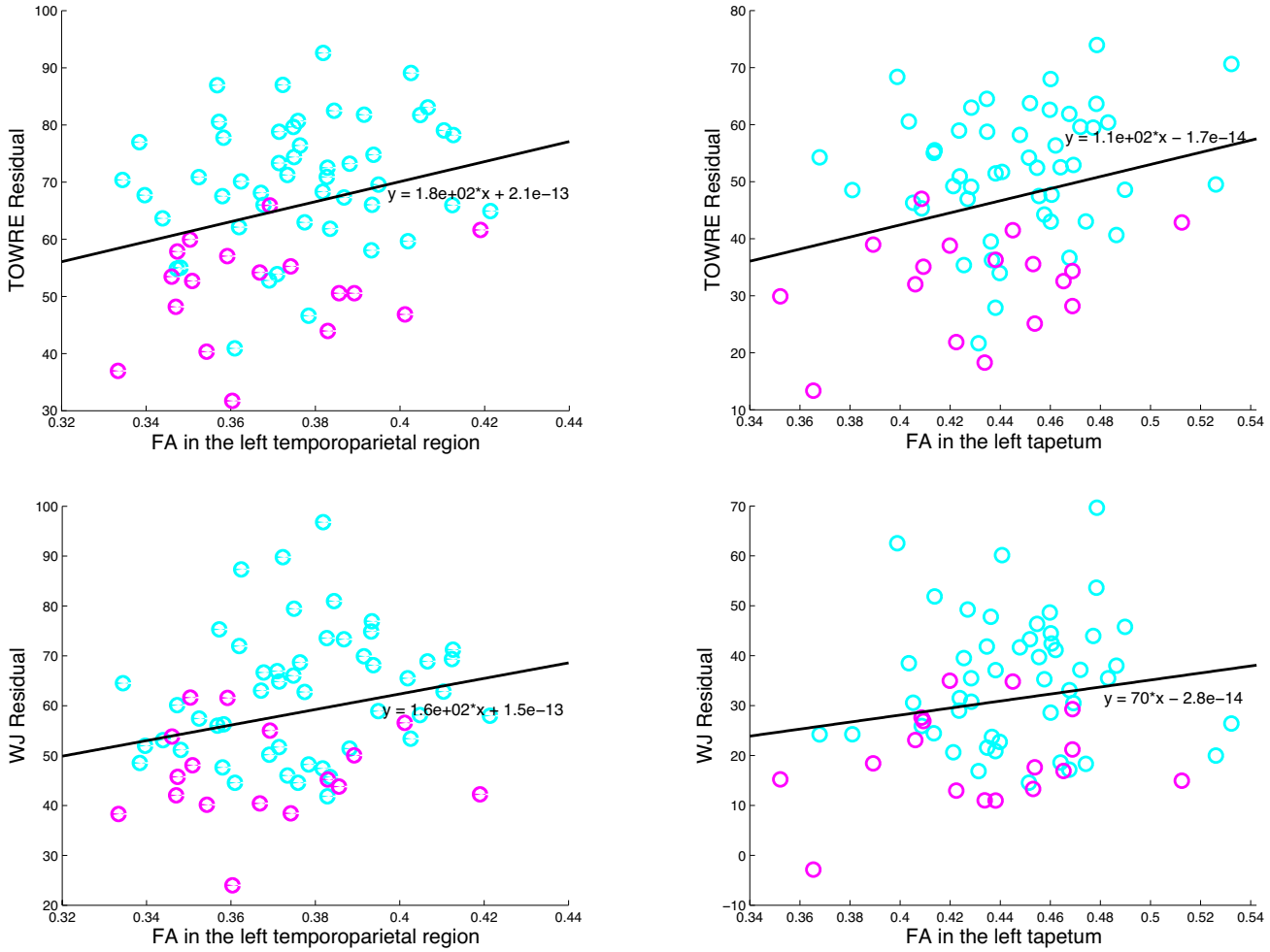

Figure 3.1 Partial residual plots of the relationship between fractional anisotropy in different white matter tracts and reading performance. (Top left) Relationship between TOWRE score and FA in the left temporoparietal region of Klingberg et al., 2000 (a 5mm diameter sphere centered at [-31, -30, 16]. (Top right) Relationship between TOWRE score and FA in left tapetum defined by ICBM atlas. (Bottom left) Relationship between WJ-III score and FA in the left temporoparietal region. (Bottom right) Relationship between WJ-III score and FA in the left tapetum. Magenta circles indicate participants with dyslexia; cyan circles indicate non-dyslexic participants.

3.3.2 Relations between diffusivity and structural volume

Next we investigated whether diffusivity was associated with volume in either the isthmus or the splenium of the posterior corpus callosum. For each region, we obtained the FA measure in the segmented region from FreeSurfer, and examined the relationship between the average FA-value and the region's volume. We found that the association between FA-value and volume was

significant in the isthmus (r = 0.37, p = 0.002; Figure 3.2B), but was not significant in the splenium (r = 0.16, p = 0.21).

Because the correlation was significant in the isthmus, we further examined other diffusivity indices (axial and radial diffusivity) in the region in an attempt to shed light on the underlying mechanisms. Specifically, we analyzed how the individual eigenvalues λ_i related to the structural volume in this region. As mentioned in the introduction of this chapter, the three eigenvalues of the anisotropic diffusion tensor are sorted by their magnitude in descending order. The first eigenvalue (λ_1) represents the diffusion coefficient along the longitudinal axis of the tract and is substantially greater than the second (λ_2) and third (λ_3) eigenvalues, which represent diffusion in two directions perpendicular to the longitudinal axis.

The second and third eigenvalues were of similar magnitude and were highly correlated with each other in both regions of the posterior corpus callosum (r = 0.90 for isthmus; r = 0.85 for splenium). Thus, we used the average of the second and third eigenvalues as a single measure of the radial diffusivity perpendicular to the longitudinal axis of the tract. The radial diffusivity ($(\lambda_2+\lambda_3)/2$) is much lower than the diffusivity along the longitudinal axis of the tract (λ_1) because the cell membrane and myelin sheaths form barriers and add impediments to the diffusion perpendicular to the tract, leading to a lower apparent diffusion coefficient (Beaulieu, 2002).

We found that the axial diffusivity (the first eigenvalue) was not correlated with the structural volume in the isthmus (r = - 0.08, p = 0.55; Figure 3.2C), but the radial diffusivity measure in this region was strongly negatively correlated with its volume size (r = -0.42, p = 0.0003; Figure 3.2D). Since radial diffusivity of fiber tracts is influenced by myelination (Beaulieu, 2002; Song et al., 2002; Song et al., 2005), our finding suggests that the reduced volume in the isthmus may have been partially due to thinner myelin of the axons. The implications of these findings are further discussed in the discussion section.

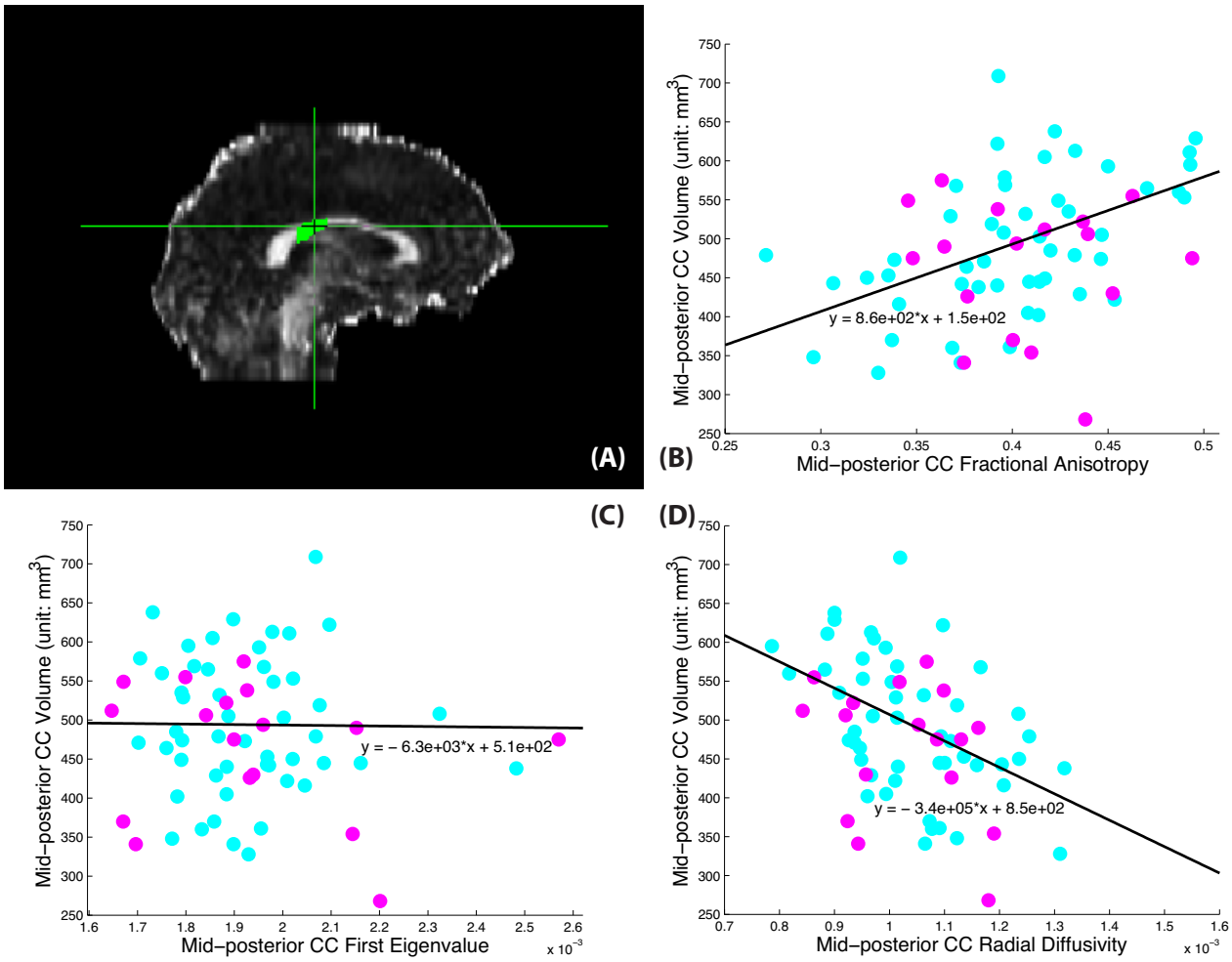

Figure 3.2 White matter diffusivity measures in the isthmus and correlation to the volume of the region. The figure shows a mask of mid-posterior corpus callosum overlaid onto an individual subject's FA image (A), correlation of the volume size with the fractional anisotropy value (B), the relationship between axial diffusivity (the first eigenvalue) and volume (C), and the relationship between radial diffusivity and volume (D). Magenta dots indicate dyslexic readers; cyan dots indicate typical readers.

3.3.3 Genetic associations

Next we investigated whether the diffusivity measures of structural connectivity were associated with risk variants of the two *KIAA0319*-related SNPs. We found that variations in SNP rs2143340, but not SNP rs9461045, were predictive of the average FA in the superior longitudinal fasciculus (SNP rs2143340: $t = 1.98$, $p = 0.052$; SNP rs9461045: $t = 1.17$, $p = 0.247$). Specifically, risk carriers of rs2143340 showed greater average FA-values in the superior

longitudinal fasciculus compared with non-risk carriers. Again, reading scores, IQ and intracranial volume were included in the models as nuisance covariates. Neither SNP was associated with FA value in the isthmus or the splenium.

Additionally, we asked whether the genetic variants predicted diffusivity in the two segments, again using ROIs segmented by FreeSurfer. Both genotypes predicted radial diffusivity in the isthmus. Specifically, the risk carriers of SNP rs9461045 showed greater radial diffusivity in the isthmus (linear regression: t = 2.06, p = 0.044), and risk carriers of SNP rs2143340 also showed marginally greater radial diffusivity in the same segment (linear regression: t = 1.93, p = 0.059). Interestingly, this association was restricted to the isthmus (the mid-posterior segment) of the corpus callosum and was not present in the splenium.

In the mid-posterior corpus callosum, both volume and radial diffusivity were associated with risk status of SNP rs9461045. Therefore we explored the hypothesis that the radial diffusivity of the mid-posterior corpus callosum was a mediator of the effect of the genetic risk on the volume of this region. To test this hypothesis, we built a mediation model with genetic risk status as an independent variable, the mid-posterior corpus callosum volume as a dependent variable, and the radial diffusivity in this region as a mediator. The mediation model was significant, with radial diffusivity of the mid-posterior corpus callosum as a significant mediator between risk status of SNP rs9461045 and the volume of mid-posterior corpus callosum (causal mediation effect = -17.9, p = 0.02).

3.4 DISCUSSION

In line with many previous studies, we found that fractional anisotropy in the left temporo-parietal region was a significant predictor of reading as measured by both the TOWRE and WJ-III composite scores. Reduced FA values were associated with poor reading performance. A similar relationship was found between the average FA value in the left tapetum and TOWRE score.

Furthermore, we analyzed the relationship between measures of volume and structural connectivity in the posterior corpus callosum, and found that the FA value was significantly correlated with the volume of the isthmus. Further

examination revealed that this correlation came from a reliable negative correlation between radial diffusivity and volume.

The left temporo-parietal region is a crucial association region for reading. In the last decade, functional activation studies have demonstrated a reduction of left temporo-parietal activation in individuals with dyslexia (Shaywitz et al., 2002; Simos et al., 2002; Temple et al., 2001), suggesting that this brain abnormality may be important in the development of reading disorders. Our findings further confirmed that the microstructural properties of white matter in the left temporo-parietal region are related to reading ability. Better readers tend to have larger fractional anisotropy measures in the left temporo-parietal region. Although the relationship between water diffusion and the tissue microstructure is not straightforward (Beaulieu, 2002; Beaulieu, et al., 2005), one possibility is that the larger FA value in good readers is related to more basic microstructural properties such as increased myelination and axonal density.

The corpus callosum, especially its mid-sagittal plane, has been a target of extensive studies and its internal structure has also been explored using diffusion tensor imaging (Darki, Peyrard-Janvid, Matsson, Kere, & Klingberg, 2012; Huang et al., 2005). Our findings based on volume measures pointed out that risk carriers have a smaller volume in the posterior corpus callosum. In an effort to explore the biological mechanism of this gross anatomical difference at the microstructural level, this chapter showed that genetic risk was associated with radial diffusivity in the mid-posterior segmentation of the corpus callosum, which presumably includes fiber tracts connecting the left and right superior parietal region (Dougherty, et al., 2007). Therefore, the effect of the genetic risk on the volume of the isthmus that we found in chapter 2 may be related to radial diffusivity in the region.

We showed that polymorphisms in the *KIAA0319* gene were marginally associated with diffusivity measures and structural volume of white matter in the mid-posterior corpus callosum. These findings suggest a potential explanation for the individual differences in anatomical structures. In the mid-posterior section of the corpus callosum, the white matter fiber tracts are aligned in a highly coherent

manner without much fiber crossing within each voxel. The diffusion perpendicular to the axons (i.e. radial diffusivity, including the second and third eigenvalues) therefore depends significantly on the properties of the insulating myelin sheath and cell membranes (Beaulieu, 2002; Song, et al., 2002; Song, et al., 2005). In fact, Song et al. demonstrated that the extent of increased radial diffusivity reflects the severity of demyelination in the corpus callosum of the mouse. Specifically, radial diffusivity increased with treatment of a specific neurotoxicant that impairs myelination, and subsequently radial diffusivity decreased with the progression of re-myelination (Song, et al., 2005).

In our study, we found that radial diffusivity, but not axial diffusivity, was significantly associated with the volume of the isthmus, and that the genetic risk associated with SNP rs9461045 was predictive of both decreased volume and higher radial diffusivity. The findings suggest that risk carriers may have a defective myelination process, leading to thinner or lower integrity myelin sheath around the callosal fibers. The result would be a less effective barrier to water diffusion and greater permeability to diffusing water, resulting in both smaller volume and greater radial diffusivity in the isthmus.

Because transcallosal pathways going through the isthmus of the corpus callosum primarily connect the left and right parietal cortex and part of the temporal lobe, the above findings in our sample suggest a functional abnormality in the left temporo-parietal region. We further examined the functional activation and functional connectivity in the temporo-parietal regions using functional neuroimaging in the next chapter.

Researchers have found many neural differences between dyslexics and control readers, and it is tempting to assume that those differences are the causes of reading problems. But it is possible that the causality goes in the opposite direction: the difference might reflect the reduced exposure of poor readers to text and reading instruction compared to normal readers and it may alter the development of a more efficient neural mechanism (networks, white matter pathways, etc.) to handle reading tasks. One way to evaluate the causal direction is to examine those markers in children (especially in pre-reading

children) who are at risk for developing dyslexia. For example, Beaulieu et al. found that regional brain connectivity in the left temporo-parietal white matter correlated with a wide range of reading ability in children as young as 8-12 years old (Beaulieu, et al., 2005). Deutsch et al. also reported the difference in FA measure between normal and poor readers in a group of children aged 7-13 years (Deutsch, et al., 2005). Raschle et al. also found that preschoolers with a family history of dyslexia tended to have less gray matter in brain regions involved in mapping the sound to their written counterpart (Raschle, Zuk, & Gaab, 2012). The consistent findings in adults and children population reduce the likelihood that the neural markers associated with reading disability are altered by experience / environmental factors.

Another potential way to evaluate the causal direction is to investigate the changes brought about by reading remediation in longitudinal study designs. Researchers have observed changes in both brain function and structure after reading remediation. For example, Keller and Just found that among poor readers, 100 hours of intensive remedial instruction resulted in significantly increased FA in the left anterior centrum semiovale (Keller & Just, 2009), and this change was associated with a decrease in radial diffusivity, but not with a change in axial diffusivity.

To conclude, we found that fractional anisotropy measure in left temporo-parietal white matter was significantly associated with reading performance, lending more support to the importance of white matter integrity in this region for reading competence. In addition, the FA measure in the isthmus (the mid-posterior section of the corpus callosum) did not contribute to reading performance but was significantly correlated with the structural volume of the isthmus. Further analysis showed that this relationship was explained by the strong negative correlation between radial diffusivity in the isthmus and its structural volume.

Our findings provide a link between previous neuroimaging and genetic results and propose a potential physiological/biological mechanism underlying

the volume variability of the posterior corpus callosum in normal and impaired readers.

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Chapter 4: Exploring Functional Neural Markers of Reading

Abstract

Functional activation markers and functional connectivity markers of reading ability and disability have been identified using functional neuroimaging techniques. In this chapter, we investigated whether any of these functional markers were associated with both reading behavior and with two risk genotypes. We collected task-related fMRI data from the same group of participants and looked for brain regions where neural activation during phonological processing was associated with reading ability. The identified region (the left supramarginal gyrus) was then used to search for functional connectivity markers. We found that the strength of functional connectivity between left and right supramarginal gyrus was significantly associated with reading ability, suggesting that this marker is an important neural underpinning of reading ability. We did not find evidence of the effect of risk genotypes on the functional markers explored in this study.

4.1 INTRODUCTION

As discussed in chapter 1, it is widely accepted that deficits in phonological awareness, the ability to recognize and manipulate the smallest units of sound in a language, are at the core of reading impairments in many dyslexic readers, who therefore have difficulty mapping written language onto spoken words (S. E. Shaywitz et al., 1998). Furthermore, growing evidence show that some dyslexic readers exhibit an orthographic coding deficit (van der Mark et al., 2009; van der Mark et al., 2011). Orthographic coding refers to the rapid recognition of the sequence of letters in words by vision and it plays an important role in the types and frequency of dyslexia's manifestations (Paulesu et al., 2001).

Based on a double dissociation found in neurological patients between reading regular pseudo-words and reading irregular, exception words, it has

been proposed that two major systems are particularly involved in skilled reading (Jobard, Crivello, & Tzourio-Mazoyer, 2003): (1) a phonological system, and (2) an orthographic system. The phonological system is likely to be composed of two components. The first component is located near the left temporoparietal junction, including the posterior superior temporal gyrus, the angular gyrus, and the supramarginal gyrus. This region is thought to be involved in phonological processing and grapheme-to-phoneme mapping, and is considered to be related to deciphering written words through a graphophonological route (Schlaggar & McCandliss, 2007; Simos et al., 2002). The second component of the phonological system includes areas in and around inferior frontal gyrus, which has been associated with articulation and naming, subvocal rehearsal (Smith & Jonides, 1999), overt segmentation of speech (Burton, Small, & Blumstein, 2000), and higher-level processes involved in the extraction of phonological units (Gandour et al., 2002). On the other hand, the orthographic system is located near the left occipito-temporal sulcus, which includes the so-called Visual Word Form Area (Cohen et al., 2000). This brain region responds automatically and rapidly to visually presented words and plays a vital role in written word recognition (McCandliss, Cohen, & Dehaene, 2003).

4.1.1 Functional activation markers of reading

The association between reading competency and task-related activation in reading-relevant brain regions has been reported by a number of functional neuroimaging studies. For instance, many studies have found that individuals with reading disabilities show reduced brain activation in the posterior temporal region and the occipito-temporal region during reading tasks (Brambati et al., 2006; Kronbichler et al., 2006; B. A. Shaywitz et al., 2002). One of the main conclusions from these studies is that dyslexia is associated with underactivation in the posterior region of the left hemisphere, which form the aforementioned temporo-parietal and occipito-temporal reading pathways in effective readers. And the underactivation of these posterior pathways in dyslexics is interpreted as reflecting an impairment with fast effortless visual word recognition (Richlan, Kronbichler, & Wimmer, 2009).

A few studies have also reported that dyslexic participants activated the left inferior frontal cortex more than controls during reading (Brunswick, McCrory, Price, Frith, & Frith, 1999; Kronbichler, et al., 2006; S. E. Shaywitz, et al., 1998). This abnormal overactivation in the frontal region or right hemisphere is usually interpreted as increased effort to compensate for the dysfunctional left posterior reading systems. But these findings of hyperactivation in the anterior region of the left hemisphere have not always been replicated (Maisog, Einbinder, Flowers, Turkeltaub, & Eden, 2008).

4.1.2 Functional connectivity markers of reading

In addition to examining task-related neural activity in different brain regions separately, an increasing number of studies are focusing on functional connectivity patterns among brain regions underlying reading processes. Since written language is a cultural artifact, it is unlikely that the human brain includes regions dedicated specifically to processing written symbols. Instead, the human brain recruits a widespread network of brain regions for reading (e.g., the visual word form system in ventral visual cortex for automatic recognition of familiar objects and symbols, the inferior frontal gyrus for subvocal rehearsal, etc.), and successful reading presumably depends on the rapid synchronization and coordination of those brain regions. Put simply, fluent reading relies not only on the adequate activation of individual reading-related cortical regions, but also on efficient communication between these processing regions (Vandermosten, Boets, Wouters, & Ghesquiere, 2012).

This hypothesis has been supported by a number of neuroimaging studies showing that reading involves a widespread network of cortical regions, predominantly in the left hemisphere, and that dyslexic readers exhibit decreased functional connectivity among those regions. In one classical account from the neurology of reading, the angular gyrus is assumed to serve a mediational role, linking orthographic output computed in extrastriate sites of the occipital lobe with lexical and linguistic representations in and around the posterior superior temporal gyrus (Geschwind, 1965). According to this account, functional

connectivity of the angular gyrus with posterior reading regions would be disrupted in dyslexic brains during processes that require phonological assembly.

A number of neuroimaging studies have demonstrated the important role of the left angular gyrus in effective functional connectivity among brain regions during reading. For instance, an early positron emission tomography (PET) study found that cerebral blood flow in left angular gyrus showed strong functional correlation with blood flow in extrastriate occipital and temporal regions during single word reading (Horwitz, Rumsey, & Donohue, 1998). A functional MRI study later found that dyslexic readers displayed a disruption in the functional connectivity among left hemisphere language regions in tasks the explicitly require phonological assembly, especially connectivity with the left angular gyrus (Pugh et al., 2000).

Individual differences in functional connectivity not only emerge in across-group comparisons between dyslexic and typical readers, but have also been shown to correlate strongly with reading performance in normal subjects. Specifically, functional connectivity between Broca's area and the left angular gyrus was reported by Hampson et al. to be highly correlated with reading performance in typical readers (Hampson et al., 2006).

The visual word form area (VWFA), which is associated with the automatic processing of visual word forms (McCandliss, et al., 2003), is also considered a crucial site in the functional connectivity network of reading. The VWFA has been shown to be part of a larger Visual Word Form (VWF) system that plays a crucial role in processing orthographic representations of visual letter-strings (van der Mark, et al., 2009; van der Mark, et al., 2011). For example, van der Mark et al. reported a significant disruption of functional connectivity between the VWFA and the left inferior and left parietal language areas in children with dyslexia (van der Mark, et al., 2011).

Finally, the left supramarginal gyrus (SMG) of the inferior parietal region has been shown to be involved in phonological and articulatory processing of words. Phonology represents one of the basic building blocks of human languages. In the case of reading, individuals must rely on phonological

representations to establish a link between symbols and meaning. While phonological processing has been shown to recruit a widespread network of cortical and subcortical areas, one region that is often identified as an important hub in the network is the inferior parietal lobule, which is divided into the angular gyrus and the SMG. More specifically, functional imaging studies have identified the left SMG as an important node in the phonological processing network (Deschamps, Baum, & Gracco, 2014). It has been reported across a number of studies that the SMG is recruited for various tasks targeting phonological processing, such as word and nonword reading, and it is preferentially activated when individuals focus on the sound of a word compared to when they focus on its meaning (Chee, O'Craven, Bergida, Rosen, & Savoy, 1999; R. L. Newman & Joanisse, 2011; S. D. Newman & Twieg, 2001). A number of studies have provided support for the SMG as a major contributor to the phonological processing (McDermott, Petersen, Watson, & Ojemann, 2003).

Based on this previous work, along with our finding that structural connectivity in the left temporo-parietal region was associated with reading performance (see chapter 3), we focused our attention on the left temporo-parietal region to look for seed region(s), which later would be used to search for functional connectivity measures. We hypothesized that during reading, better readers would show greater activation in, and greater functional connectivity between, brain regions that contribute to phonological processing than would poor readers.

After a seed region was identified based on activation during phonological processing, we used that region as a seed to search for functional connectivity markers related to reading behavior. Specifically, we looked for evidence that: 1) functional activity in the target region varies with that in the seed region during reading processing; 2) the strength of functional connectivity between the target and seed regions is associated with reading ability. In addition, we further explored whether any of the functional markers were related to the two genotypes we studied. To our knowledge, little work has been done to investigate

the effect of dyslexia risk genes on reading-related functional connectivity markers.

4.2 METHODS

4.2.1 Participants

The functional data described in this chapter were collected from the same set of subjects described in chapter 2. See section 2.2.1 in chapter 2 for a detailed description of our sample. Nine out of 68 participants were excluded from all the analyses in this chapter because four participants' functional images (from one or more runs) were badly distorted after preprocessing steps, and physiological data were not collected for another five participants. Therefore, 59 participants were included in these analyses.

4.2.2 Polymorphism genotyping

See section 2.2.2 in chapter 2 for a description of the polymorphism genotyping process.

4.2.3 Stimuli and tasks

We followed Shaywitz et al. (S. E. Shaywitz, et al., 1998) and used three tasks ordered hierarchically to tap into subcomponents of reading (see Figure 4.1). In each trial, two stimuli were presented simultaneously, one above the other, and the participant was asked to make a judgment about the pair. There were three conditions: judge whether the orientations of two sets of lines matched, judge whether the case of two sets of letters matched, and judge whether two nonwords rhymed or not. (i) At the lowest level, the line orientation judgment task requires visual-spatial processing but makes no orthographic demands. (ii) At an intermediate level, the letter-case judgment task adds an orthographic processing demand but makes no phonologic demands. (iii) At the highest level, the nonword rhyming (NWR) task adds a phonologic processing demand, requiring the transcoding of the letters (orthography) into phonologic units and then a phonological analysis of those units. These tasks were presented in 4 runs of 5 min and 12 s each while functional magnetic resonance images (fMRI) were collected. Each condition was repeated three times in each run, with 4 trials in each block. Thus, the analysis included 12 repeated blocks

per task. Stimulus pairs in all three tasks were presented at a rate of 1 every 5.5 seconds, a rate that previous studies suggested was comfortable for dyslexic readers. Visual stimulus presentation and response collection was controlled by E-prime software. Stimuli were presented through a backlit projection screen, visible to the subject by a mirror mounted on the top of the head coil. Responses were collected using an MRI-compatible response claw. To familiarize participants with the task, they were given a short practice version of the task outside the scanner.

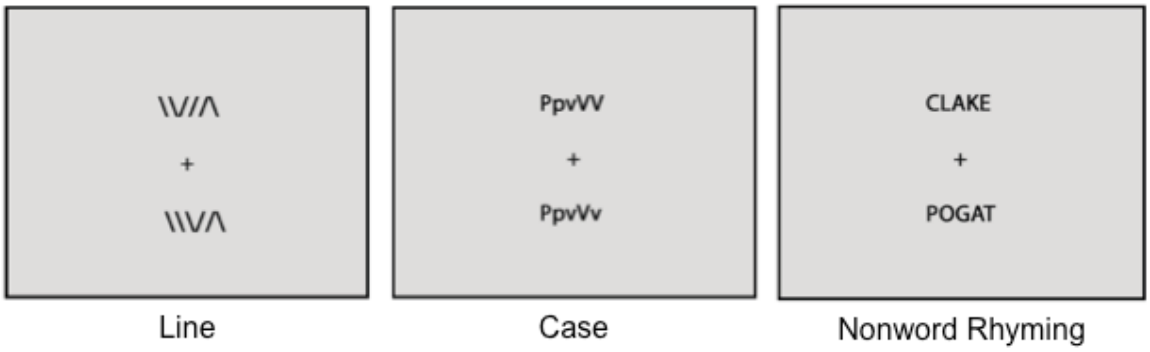

Figure 4.1 Illustrative stimuli for three tasks in the scanner: line, letter-case, and nonword rhyming.

4.2.4 Functional Imaging acquisition

The fMRI experiment was conducted on a 3 Tesla MRI scanner at the University of Michigan's Functional Magnetic Resonance Imaging Laboratory. A standard head coil was used and head movements were minimized by cushions. Functional MR data were acquired using an echo-planar imaging pulse sequence to measure blood-oxygen-level-dependent (BOLD) T2* contrast. Other acquisition parameters were as follows: TR = 2000 millisecond, TE = 30 millisecond, field of view = 259 mm, and slice thickness = 4 mm. Each volume contains 43 axial slices. The first five volumes of each run were discarded. A T1-weighted anatomical image was acquired after the functional scans. The field of view was 259 mm, voxel size was 1 mm × 1 mm × 1.2 mm (123 axial slices), TR (repetition time) was 9 msec, TE (echo time) was 1.8 msec, flip angle was 15°, and the whole structural scan lasted about 6 minutes.

4.2.5 Behavioral data analysis

Reading accuracy and reaction time inside the scanner were computed and associated with the reading assessment results outside the scanner.

4.2.6 Functional data pre-processing and activation analysis

The functional data were processed using SPM8 (Wellcome Department of Cognitive Neurology, London, United Kingdom, http://www.fil.ion.ucl.ac.uk) on MATLAB (R2013a). The functional images were first reconstructed and corrected for physiological parameters and slice timing, and then were realigned to the mean volume to correct for head movement. Each participant's T1 anatomical scan was coregistered with the functional images and then segmented into gray matter, white matter, and cerebral spinal fluid. The gray matter was normalized into the default gray matter probability template in standard MNI space, and the acquired normalization parameters were used to normalize the realigned functional images for each participant with a spatial resolution of 3 mm × 3 mm × 3 mm. The resulting functional images were then smoothed with an 8 mm full-width-half-maximum Gaussian kernel.

At the individual level, we first used a standard general linear model (GLM) approach to estimate the neural activation in response to experiment conditions (i.e., line, letter-case, non-word rhyming) in contrast to the baseline fixation. The model included separate regressors for each of the conditions convolved with a canonical hemodynamic response function. We also included an additional covariate to model the 2-second instructions at the beginning of each block, as well as the six rigid-body movement parameters as nuisance covariates. Individual contrast images were created for primary contrasts of interest between conditions: *NWR (non-word rhyming)* > *Case* and *Case* > *Line*. These individual contrast images were entered into a second-level random effects group analysis. The left supramarginal gyrus (SMG) identified in the *NWR* > *Case* regression analysis served as a seed region in the subsequent psychophysiological interaction analysis of functional connectivity.

4.2.7 Psychophysiological interaction analysis of functional data

We used the Psychophysiological Interaction (PPI) approach to identify brain regions that showed task-dependent functional connectivity with the seed

regions. The goal of Psychophysiological interaction analyses is to determine the degree to which neural physiology in the seed region and target region(s) co-vary as a function of experiment conditions (Friston et al., 1997; Gitelman, Penny, Ashburner, & Friston, 2003). When examining the structural connectivity markers (see chapter 3), we found that white matter integrity in the left temporo-parietal region was significantly associated with reading performance. Based on our findings on structural connectivity, we focused on the left temporo-parietal region for our functional connectivity analysis. As mentioned in the last section, we first performed a whole brain regression analysis on the functional activation under NWR > letter-case contrast to search for brain regions where good readers would show greater activation under phonological processing than poor readers. The neural activity in the left SMG was associated with reading competency (see more details in results section 4.3.2). We then used the left SMG as a seed region of interest for functional connectivity analysis (i.e., NWR > letter-case). The seed region was defined by constructing a spherical volume of interest with a radius of 5mm centered on the group-level peak voxel (MNI coordinates [-63, -36, 33]). For this region of interest, the first eigenvector of the BOLD time series within the region was extracted, and the time-series served as the physiological variable (*y*).

Next, a PPI model was constructed to find brain regions showing different patterns of connectivity with the seed region as a function of two different tasks (rhyme and letter-case). In each participant, the psychological variable (*p*) was defined by the *non-word rhyming* > *letter-case* contrast, and the interaction variable (*ppi*) was constructed by taking the product of *p* and the deconvolved time series *y*. The interaction term tests whether the connectivity between the seed activity and the target activity was modulated by the psychological context. In other words, it can be interpreted as the difference in the degree to which target activity is explained by seed activity in the rhyme versus letter-case condition. The parameter estimate of *ppi*, which represents this degree of modulation, is henceforth referred to as the PPI parameter estimate. The

individual PPI parameter estimate images were then carried to the second level for random effects analysis.

At the second level analysis, a whole-brain regression analysis was performed to identify brain regions whose functional connectivity or co-variance with the seed region varied as a function of the tasks (e.g., NWR vs. letter-case). Next, linear regressions were performed to determine whether the strength of the connectivity between the seed and the identified region was associated with reading performance.

We further tested whether individual differences in functional connectivity PPI parameter estimates between the seed and target regions were associated with the two genetic variants we genotyped in our study. We hypothesized that functional connectivity between left SMG of the inferior parietal region and other reading-related brain areas (e.g., the posterior superior temporal gyrus, angular gyrus, the angular gyrus, VWFA) would be associated with reading competency, and possibly with genetic risk as well.

4.3 RESULTS

4.3.1 In-scanner task performance

Reaction and accuracy for the tasks performed inside the scanner are reported in table 4.1. For the non-word rhyming (NWR) condition, which involves phonological processing, the reaction time was significantly correlated with out-scanner reading assessment (TOWRE: *Pearson r* = -0.59, $p < 0.001$; WJ-III: *Pearson* r = -0.67, p < 0.001). Specifically, participants with higher reading assessment scores responded faster in the nonword rhyming task. The relationship was significant after controlling for IQ (TOWRE: $t = -4.63$, $p < 0.001$; WJ-III: $t = -6.03$, $p < 0.001$). The accuracy measures of this condition showed a similar pattern when correlated with assessment results (TOWRE: *Pearson r* = 0.50, $p < 0.001$; WJ-III: *Pearson r* = 0.48, p < 0.001), and the results remained significant after controlling for IQ (TOWRE: $t = 3.08$, $p = 0.003$; WJ-III: $t = 2.88$, $p = 0.005$). Good readers made faster judgments and fewer errors in the non-word rhyming condition.

Table 4.1 Mean accuracy and reaction time in each condition inside the scanner. Standard deviations are reported in the parentheses.

Measures	Line	Case	Nonword rhyming
Accuracy (%)	0.97 (0.03)	0.97 (0.03)	0.91 (0.08)
Control	0.97 (0.03)	0.97 (0.03)	0.93 (0.06)
Dyslexic	0.97 (0.03)	0.97 (0.02)	0.83 (0.07)
Reaction Time (ms)	1526 (314)	1813 (337)	1844 (485)
Control	1505 (316)	1762 (325)	1671 (345)
Dyslexic	1589 (310)	1967 (335)	2364 (480)

4.3.2 Functional activation selective to letter-case and rhyme conditions

The main goal of the current study was to search for functional activation or functional connectivity markers that are related to reading performance or genetic risk. As a first step to achieve this goal, we identified regions that were selectively activated in phonological (rhyming > letter-case) processing, and that were associated with reading performance as promising seed regions for subsequent PPI analysis.

Table 4.2 Suprathreshold clusters and local maxima information in contrasts of interest.

Contrast	Cluster size (voxel)	Peak Z	Peak MNI coordinate	Peak label
Rhyme > Case				
	1008	7.30	[-45, 42, 0]	Frontal_Inf_Tri_L
	56	7.17	[21, -69, -30]	Cerebelum_6_R
	50	6.10	[0, 24, 48]	Supp_Motor_Area_L
	34	6.01	[-12, -3, 18]	Caudate_L
	298	5.98	[51, 48, -9]	Frontal_Inf_Orb_R
	133	5.98	[-54, -54, -15]	Temporal_Inf_L
	76	5.31	[-6, 6, 72]	Supp_Motor_Area_L
	117	4.98	[9, 3, 9]	Caudate_R
	85	4.75	[-15, -96, -6]	Calacrine_L
	99	4.73	[-30, -63, 39]	Parietal_Inf_L
	79	4.68	[-9, -12, 9]	Thalamus_L
	32	4.55	[-42, 0, 54]	Precentral_L
	110	4.36	[36, 54, -33]	Undefined
Case > Line				
	131	Inf	[24, -102, -9]	Occipital_Inf_R
	120	Inf	[-24, -99, -12]	Occipital_Inf_L
	23	7.23	[-36, -90, -18]	Occipital_Inf_L
	236	6.80	[-39, -75, -12]	Occipital_Inf_L

82	5.99	[36, -51, -21]	Fusiform_R
53	5.50	[-27, -63, 45]	Parietal_Sup_L
233	5.21	[-9, -72, 12]	Calcarine_L
101	4.68	[-39, -15, 57]	Precentral_L
28	4.42	[36, -63, 42]	Angular_R
59	3.97	[3, 0, 69]	Supp_Motor_Area_R
38	3.97	[-36, 9, 30]	Front_Inf_Oper_L
28	3.73	[-15, -15, 72]	Precentral_L

Table 4.2 presents brain areas that were significantly activated by the *NWR* > *Case* contrast (phonological) and the *Case* > *Line* contrast (orthographic). Both phonological and orthographic processing activated a widespread network of regions, with the left inferior frontal gyrus and left inferior parietal lobule as important regions for phonological processing. To further explore the relationship between reading ability and the strength of activation in those brain regions, we performed a whole-brain regression analysis in search of regions where the neural activation level was associated with reading performance. The activation level in the left SMG of the inferior parietal was found to correlate with reading performance ($\alpha < 0.005$, voxel peak at [-63, -36, 33]).

To show this relationship, neural activation in this region under the *NWR* > *letter-case* contrast was extracted and entered into a multiple regression model to predict reading scores, with IQ included as a nuisance covariate. The activation in the left SMG was a significant predictor of reading scores (TOWRE: $t = 2.81$, $p = 0.007$; WJ: $t = 2.77$, $p = 0.008$). The partial residual plot of the activity in the left supramarginal gyrus in predicting reading scores is shown in Figure 4.2. None of the other brain regions that showed extensive activation during phonological or orthographic processing significantly predicted reading performance. The results showed that although these regions were reliably activated in their corresponding contrast (e.g. NWR > Case), the activation level in those regions was not a strong predictor of reading ability.

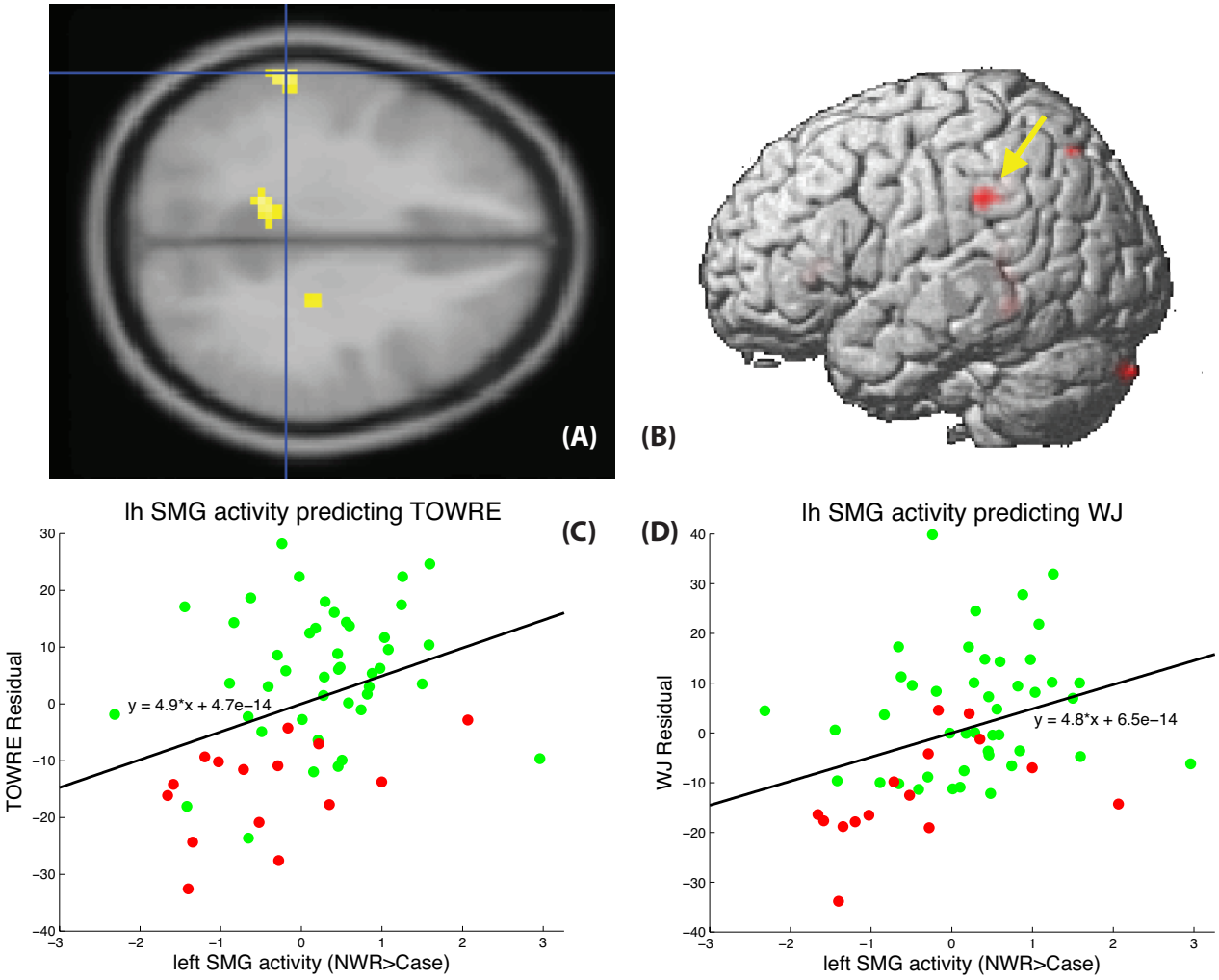

Figure 4.2 (A & B) Results of a whole-brain regression analysis of *NWR* > *Case* activation against TOWRE score. Activation in the left supramarginal gyrus (SMG) (peak voxel at coordinate [-63, -36, 33]) was one of the best predictors of TOWRE score. The yellow arrow in B points at the left SMG; the threshold was lowered to $p = 0.05$ for visualization. (C & D) The lower panels show partial residual plots of significant relationships between left SMG activity and two reading scores. Red dots indicate participants who were diagnosed as dyslexic. Green dots indicate non-dyslexic participants.

4.3.3 Functional connectivity from left SMG predicting reading behavior

Neural activity in the supramarginal gyrus in response to phonological processing was significantly associated with reading performance. Therefore, we used this region as a seed to search for functional connectivity markers. We examined the functional connectivity arising from this region using PPI analysis, and several brain regions were revealed to be effectively connected with the

seed region (see Table 4.3; only results from the positive contrast are shown; the reverse contrast showed no significant regions in the whole brain). Functional connectivity between each of these regions and the seed region varied as a function of phonological processing demand (hence the significant interaction between the psychological factor and physiological activity). Specifically, the seed and target regions showed stronger connectivity when phonological processing was required than when it wasn't.

Next we examined whether the degree of effective connectivity between the SMG and target regions was related to individual's reading ability. If such effective connectivity supports grapheme-to-phoneme mapping / correspondence, we would expect greater connectivity to be associated with better reading performance. Using Pearson correlation and multiple regression models, we assessed whether the PPI parameter estimates, an estimate of functional connectivity, predicted reading scores.

The analyses revealed that PPI parameter estimates in the left superior parietal region correlated with reading ability (TOWRE: $r = 0.274$, $p = 0.036$; WJ: $r = 0.272$, $p = 0.037$). This showed that good readers showed stronger connectivity between the left SMG and the left superior parietal region during phonological processing than did poor readers. The relationship was marginally significant after including IQ as a covariate to predict reading (TOWRE: $t = 1.837$, $p = 0.072$; WJ: $t = 1.816$, $p = 0.075$). For the PPI parameter estimates in the right inferior parietal region, the correlation with TOWRE was marginally significant ($r = 0.237$, $p = 0.071$) but the correlation with WJ was not ($r = 0.196$, $p = 0.138$). The results remained similar when IQ was included as a covariate (TOWRE: $t = 1.845$, $p = 0.070$; WJ: $t = 1.479$, $p = 0.145$). The same analyses were performed for the PPI parameter estimates in the right SMG, and the results revealed significant correlation with reading performance (TOWRE: $r = 0.465$, $p < 0.001$; WJ: $r = 0.370$, $p = 0.004$), and the predictive power remained significant after including IQ as a covariate (TOWRE: $t = 3.211$, $p = 0.002$; WJ: $t = 2.206$, $p = 0.032$; see Figure 4.3). This showed that good readers exhibited stronger connectivity between the left and right SMG during phonological processing than

poor readers. This finding implies that the functional connectivity between the left and right SMG might be an important neural underpinning of reading ability.

Table 4.3 Suprathreshold clusters and local maxima information in PPI analysis of NWR > Case contrast using the left supramarginal gyrus as seed region.

Contrast	Cluster size (voxel)	Peak Z	Peak MNI coordinate	Peak label
PPI (Interaction)				
	103	3.48	[-9, -36, 3]	Sub-lobar / Extra-Nuclear
	79	3.48	[21, -6, -30]	ParaHippocampal_R
	71	3.47	[-9, -48, 63]	Precuneus_L
	92	3.30	[-18, -15, -6]	Sub-lobar / Extra-Nuclear
	53	3.17	[60, -36, 57]	Supramarginal_R
	35	3.16	[-15, -57, 51]	Parietal_Sup_L
	70	3.15	[30, -51, 54]	Parietal_Inf_R

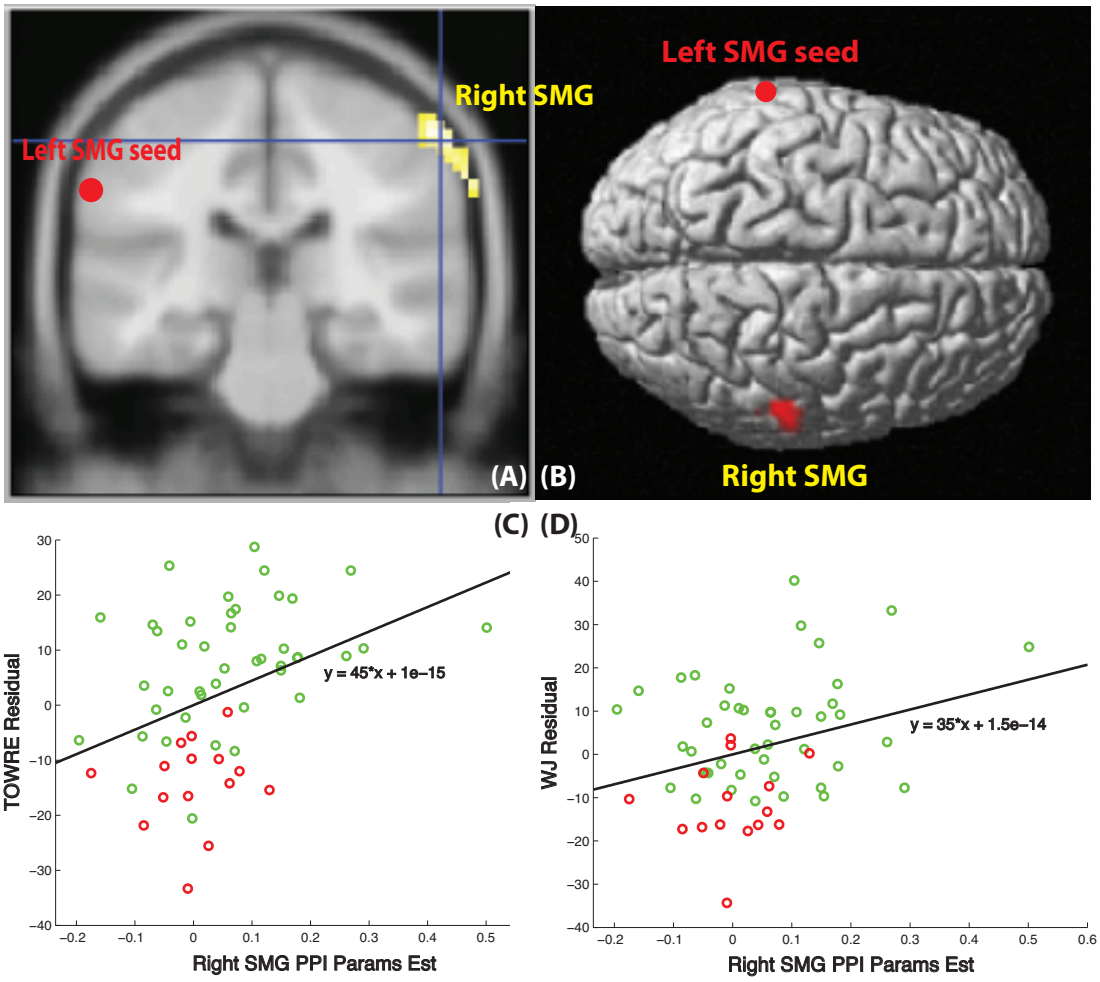

Figure 4.3 The top panel shows the location of left and right supramarginal gyri (SMG) in a coronal view (A) and a render view of the brain from the top (B). The bottom panel shows partial residual plots of relationships between functional connectivity parameter estimates (between bilateral supramarginal gyri during phonological processing) and TOWRE (C) and WJ (D). Red circles indicate participants who were diagnosed as dyslexic. Green circles indicate non-dyslexic participants.

4.3.4 Relationship between functional and structural connectivity markers

One interesting finding was that the functional connectivity between the left and right SMG significantly predicted reading performance. Given our previous findings that the structural volume and microstructural properties of the posterior corpus callosum (especially in the mid-posterior corpus callosum) were good predictors of reading ability, we further explored the relationship between the functional connectivity measure and the structural measures in chapter 2 and chapter 3. We hypothesized that there would be a significant relationship between functional connectivity (PPI parameter estimate) between bilateral SMG during phonological processing and the structural properties (volume and diffusivity) of the posterior corpus callosum. However, we did not detect any significant relationships between the functional markers and the structural markers (correlation with mid-posterior corpus callosum volume: $r = 0.101$, $p = 0.451$; with mid-posterior corpus callosum FA: $r = -0.022$, $p = 0.870$).

4.3.5 Relationship between functional markers and genetic risk

Finally, we performed multiple regressions on the functional activation in the left SMG as well as functional connectivity markers, with average functional markers (activation level or PPI parameter estimate) as the dependent variable and genetic risk as an independent variable. We also included IQ and reading scores as nuisance covariates. The analyses showed that neither of the two genotypes significantly predicted any of the functional connectivity markers: from the left SMG to the right SMG (rs9461045: $t = -0.873$; $p = 0.662$; rs2143340: $t = -0.084$; $p = 0.933$), from left SMG to left superior parietal cortex (rs9461045: $t = 0.510$; $p = 0.612$; rs2143340: $t = -0.357$; $p = 0.723$), from left SMG to right inferior parietal cortex (rs9461045: $t = -0.002$; $p = 0.998$; rs2143340: $t = -0.468$; $p =$

0.642). Likewise, genotypes were not associated with functional activation in the left SMG either (rs9461045: t = -0.304; p = 0.763; rs2143340: t = -1.723, p = 0.091).

4.4 DISCUSSION

In this chapter, we examined neural markers of reading based on functional activation and functional connectivity. We found that reaction time and accuracy of in-scanner non-word rhyming was reliably correlated with out-scanner reading assessment results. Using functional activation analysis, we found that the left inferior frontal gyrus, inferior temporal gyrus, inferior parietal gyrus and supplementary motor area were significantly activated during phonological processing, while the bilateral inferior occipital and the calcarine sulcus were extensively activated during orthographic processing.

Among all the regions, the supramarginal gyrus (SMG) in the left inferior parietal lobule was the only region whose neural activity exhibited a significant association with reading performance. Using the left SMG as a seed region, we found that several brain regions showed prominent functional connectivity with the seed during phonological processing. We further explored the regions located in the temporo-parietal region, including regions in the left superior parietal lobule, right inferior parietal lobule and right SMG. The analyses revealed that the connectivity arising from the left SMG to the right SMG was a strong predictor of reading performance, followed by connectivity strength from left SMG to left SPL. The connectivity measure between the left SMG and right IPL during phonological processing was not associated with reading performance.

Prior work already established the crucial role of the SMG in the phonological and articulatory processing of words, and it has been shown to be preferentially activated when individuals focus on the sound of a word compared to when they focus on its meaning. Not surprisingly, we found good readers tended to activate this region to a greater extent during phonological processing (nonword rhyming > letter-case contrast) than poor readers.

More interestingly, when this region was used as a seed to search for brain regions showing functional connectivity with the seed, the strength of

connectivity from the seed to a few identified regions showed association with reading performance. For example, good readers showed stronger connectivity between the left and right SMG during phonological processing than poor readers. This suggests that the left SMG is an important hub for phonological processing in the brain. The findings in the functional connectivity differences also add to the growing recognition that dyslexia is a disconnection syndrome (Boets et al., 2013; Vandermosten, et al., 2012). Furthermore, in addition to connections among reading-related regions in the left hemisphere, our findings once again point out the importance of inter-hemispheric connection for reading processing.

The left inferior frontal gyrus was also found to be extensively activated during phonological processing in our study. This finding is not surprising given that this area is involved in phonological rehearsal / segmentation. But we did not observe a correlation between neural activity in this region and reading ability. This might suggest that neural activity in this region is intact in poor readers; perhaps it is the coordination and synchronization among widely distributed cortical and subcortical regions that is impaired.

We did not observe any effect of SNP rs9461045 or SNP rs2143340 on the functional activation and connectivity markers. It is possible that the functional markers are associated with other genetic risk factors that are not included in the current study, or that we did not have enough power to detect the effect due to our sample size. Another possibility is that the functional connectivity markers are more transient and malleable compared to relatively hard-wired anatomical structures or properties, and therefore are more susceptible to environmental influences such as quality and quantity of reading instruction or time spent on reading. The functional measures are also likely to be noisier than the structural measures which would also make it harder to identify genetic associations, especially given the sample size of our study.

This study did not explore functional connectivity during orthographic processing. Although the prevalence of phonological deficits is higher than orthographic impairment, there is evidence that orthography plays a significant

role in some cases of dyslexia and may influence how it manifests itself (Paulesu, et al., 2001). A natural next step would be to explore the network underlying orthographic processing. Specifically, we hypothesize that the VWFA is a promising hub in the orthographic processing network.

In conclusion, we have shown that the degree of functional connectivity between the left supramarginal gyrus (SMG) and the right SMG during phonological processing is associated with reading performance, especially performance on the TOWRE. Good readers exhibited stronger connectivity between the bilateral SMG in tasks requiring phonological assembly. This suggests that the left SMG may be an important hub in the phonological processing network, and that bilateral SMG connectivity might be an important marker for reading competency.

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Chapter 5: General Discussion

5.1 SUMMARY OF RESULTS AND SIGNIFICANCE

The purpose of this dissertation was to investigate the neural and genetic substrates of reading ability and disability using an imaging genetics approach. We collected neuroimaging data from different modalities to examine (1) how the individual differences in structural and functional neural markers predicted reading behavior, (2) how the genetic risk status of two single nucleotide polymorphisms (SNPs) predicted individual differences in the neural markers, and (3) the relationships between neural markers collected across different modalities.

Chapter 2 examined prominent structural brain markers and their relationship with reading performance and with two genetic polymorphisms. Using factor analysis to reduce the dimensionality of the data, we found that posterior corpus callosum volume was a significant predictor of reading performance, even after controlling for IQ, total brain size, and other structural markers. Furthermore, this posterior corpus callosum factor showed a significant association with the genetic risk status of SNP rs9461045 in the *KIAA0319* dyslexia-susceptibility gene. Specifically, posterior corpus callosum volume tends to be greater in good readers and in non-risk carriers of SNP rs9461045. The risk allele of this SNP reduces the expression level of the *KIAA0319* gene, which has been shown to play a role in neuronal migration and axon guidance. The results suggest the possibility that the risk allele may impair the coordinated changes required in the development of the corpus callosum, and therefore lead to a less developed corpus callosum in risk carriers.

By linking variation in corpus callosum volume with both reading performance and genetic risk, we have identified a potential endophenotype for reading. The results suggest that one way how the *KIAA0319* gene may affect reading behavior is through influencing the corpus callosum and associated interhemispheric communication.

In chapter 3, we looked into white matter microstructural properties using diffusion tensor imaging, and again investigated their relationship with both reading behavior and genetic risk in the same group of participants. We found that fractional anisotropy (FA, a measure of white matter integrity) in the left temporo-parietal white matter was associated with reading performance. Furthermore, the volume of the isthmus (mid-posterior corpus callosum) was significantly correlated with FA in this region, and this correlation came from a reliable negative correlation between the volume of the isthmus and its radial diffusivity. Additionally, genetic risk associated with rs9461045 was predictive of both decreased volume and increased radial diffusivity. Therefore, we speculate that the risk carriers might have a defective myelination process, leading to thinner or less integral myelin sheaths around the callosal fibers. This provides a potential biological mechanism underlying the volume variability in the posterior corpus callosum in good and poor readers.

Finally in chapter 4, we used functional neuroimaging along with three hierarchically ordered tasks to determine whether any functional activation or connectivity markers were associated with reading performance or genetic risk. Neural activity in the left supramarginal gyrus (SMG) during phonological processing was revealed to be associated with reading ability, with good readers showing more activation in the region than poor readers. Furthermore, psychophysiological interaction (PPI) analyses revealed that functional connectivity between left and right SMG was associated with reading performance, with good readers showing stronger connectivity between left and right SMG in tasks requiring phonological assembly, suggesting that synchronizing activity between the two parietal cortices (perhaps for phonological

processing) is an important component of reading. The findings also suggest that the left SMG is an important hub in the phonological processing network.

Overall, using an imaging genetics approach, our findings extend previous research on the neural and genetic basis of reading and literacy, and illustrate the promise of combining behavioral methods, neuroimaging, and genetic analyses to gain insight into a complex cognitive process at multiple levels of analysis.

5.2 LIMITATIONS AND FUTURE DIRECTIONS

Some limitations of the current studies should be acknowledged. First, the sample size in the current study is very small by the standards of genetics research, where sample sizes of hundreds or even thousands are needed because the effect size of each SNP in complex disorders is typically very small. The results from our genetic-neural analyses should therefore be interpreted with caution and should be replicated with independent samples. It should be noted that in order to increase statistical power, we focused on testing whether two specific single nucleotide polymorphisms were associated with neural measures of reading, and we also enriched our sample by recruiting disproportionately greater number of dyslexic readers and risk carriers. However, future studies with a larger sample size will allow more statistical power to detect the effects of a greater number of prominent dyslexia risk genes on reading-related neural measures.

It is also important to keep in mind that reading disability is likely a heterogeneous disorder with different symptoms, causes, and genetic risk factors (Castles, Datta, Gayan, & Olson, 1999). A further step in a mechanistic understanding of reading disability is to know how different genetic variants correspond to specific morphological or functional alterations in the brain. However, due to the scope and sample size of the current study, we were only able to explore two prominent genetic variants related to a single susceptibility gene. Future large-scale imaging genetics studies simultaneously focusing on

various aspects of structural and functional variations are needed to explore alternative gene-brain-behavior pathways and to investigate how the different pathways relate to each other.

This dissertation also did not investigate the many important environmental factors that influence reading and that interact with genetic factors. There is widespread support for the notion that environmental and biological factors are both important determinants of a child's reading and linguistic ability (Eckert, Lombardino, & Leonard, 2001). Since reading is a skill acquired through long-term instruction and practice, it is natural that environmental factors play a major role in determining reading achievement. Previous work has shown that reading performance is influenced by environmental variables such as time spent on reading, quality of reading instruction, peer and family influences, and socioeconomic status (Olson, Keenan, Byrne, & Samuelsson, 2014).

Recent functional and structural neuroimaging studies indicate that neural markers can be changed by reading intervention and training. For instance, Temple et al. reported that after a remediation program focused on auditory processing and oral language training, dyslexic children showed increased activity in left temporo-parietal cortex and left inferior frontal gyrus, bringing neural activation in these regions close to that seen in normal-reading children (Temple et al., 2003). Another study found that intensive remedial instruction resulted in significantly increased white matter integrity in the left anterior centrum semiovale in young poor readers (Keller & Just, 2009).

Genetic effects on reading ability are also modulated by environmental factors. For example, Taylor et al. compared monozygotic and dizygotic twins to obtain an estimate of genetic variance in reading achievement and found that teacher quality modulates the genetic effects on early reading achievements (Taylor, Roehrig, Hensler, Connor, & Schatschneider, 2010). Another study by Friend et al. reported that genetic influence was higher and environmental influence was lower among children whose parents had a high level of education

when compared with children whose parents had a lower level of education (Friend, DeFries, & Olson, 2008). Relating this to our study, most of our subjects were recruited from the Ann Arbor area, and therefore represent a group with higher education and more supportive environment compared to the national average. This might have facilitated our search for genetic associations with structural markers.

All the findings in our studies raise interesting questions regarding whether the neural properties we measured can be changed by experience. However, the cross-sectional nature of our study and the lack of environmental measures make it impossible for us to infer the effect of environmental factors on neural measures during development.

The current studies also did not allow us to make direct, causal inferences about the mechanisms by which dyslexia risk genes influence the brain and the brain influences reading behavior. Future studies using longitudinal designs along with reading interventions are needed to make stronger inferences about the mechanisms involved. It is also possible to “borrow” findings from studies using animal models, especially for understanding the path from gene to brain. Generally it is very difficult to model dyslexia with animal models because it is a very complex and uniquely human trait, but animal models do make it possible to see the effect of a specific gene on the brain (e.g., knockdown of the *KIAA0319* gene led to reduced corpus callosum size in male rats).

Finally, it is important to note that our study targeted a population of white European descent because a large majority of prior behavioral genetic studies were carried out in this population. The findings based on samples of European ancestry do not necessarily apply to other populations due to the genetic variation that exists between populations. Therefore further studies are required to look at the genetic-neural relationship in other ethnic and racial groups in order to determine whether our findings can be generalized to other populations.

5.3 TRANSLATIONAL RELEVANCE

Understanding the gene-brain-behavior pathways involved in reading can add to our basic understanding of the neurobiological mechanisms underlying reading behavior. However, this knowledge may also have a translational impact in the near future, leading to improved early screening and biological-based prevention and treatment programs for reading disabilities.

The approach adopted in this project could also help in the search for other susceptibility genes of dyslexia. Clearly, the pathway from genotype to the dyslexic phenotype(s) is long and complicated. Not surprisingly then, only a few candidate susceptibility genes have been identified, and together they make only a small contribution to increased risk and leave a large amount of phenotypic variation unaccounted for. Presumably, many other susceptibility genes are still out there to be identified. Our results are consistent with the idea that neural markers are more strongly associated with genetic variants than are behavioral measures, and so they may provide a more powerful way to identify genetic risk factors.

To conclude, the goal of this dissertation was to add to our understanding of the neural and genetic substrates of reading. Most of our findings pointed to the importance of efficient connection and coordination among widely distributed brain regions in a reading-related network. We hope these studies make an important contribution to our understanding of the neurobiological substrates of reading, and will eventually help lead to improved screening, prevention and treatment programs for reading disability.

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