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Neuroimaging genetic associations between *SEMA6D*, brain structure, and reading skills

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ABSTRACT

Specific reading disability (SRD) is defined by genetic and neural risk factors that are not fully understood. The current study used imaging genetics methodology to investigate relationships between *SEMA6D*, brain structure, and reading. *SEMA6D*, located on SRD risk locus DYX1, is involved in axon guidance, synapse formation, and dendrite development. *SEMA6D*'s associations with brain structure in reading-related regions of interest (ROIs) were investigated in a sample of children with a range of reading performance, from sites in Connecticut, CT (n = 67, 6–13 years, mean age = 9.07) and San Francisco, SF (n = 28, 5–8 years, mean age = 6.5). Multiple regression analyses revealed significant associations between *SEMA6D*'s rs16959669 and cortical thickness in the fusiform gyrus and rs4270119 and gyrification in the supramarginal gyrus in the CT sample, but this was not replicated in the SF sample. Significant clusters were not associated with reading. For white matter volume, combined analyses across both samples revealed associations between reading and the left transverse temporal gyrus, left pars triangularis, left cerebellum, and right cerebellum. White matter volume in the left transverse temporal gyrus was nominally related to rs1817178, rs12050859, and rs1898110 in *SEMA6D*, and rs1817178 was significantly related to reading. Haplotype analyses revealed significant associations between the whole gene and brain phenotypes. Results suggest *SEMA6D* likely has an impact on multiple reading-related neural structures, but only white matter volume in the transverse temporal gyrus was significantly related to reading in the current sample. As the sample was young, the transverse temporal gyrus, involved in auditory perception, may be more strongly involved in reading because phonological processing is still being learned. The relationship between *SEMA6D* and reading may change as different brain regions are involved during reading development. Future research should examine mediating effects, use additional brain measures, and use an older sample to better understand effects.

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SEMA6D; reading ability; brain structure; neuroimaging; genetics

Introduction

Specific reading disability (SRD), diagnosed when individuals have significant difficulty with reading words or text, affects about 7% of the population, putting them at risk for poor academic performance (Hulme & Snowling, 2016). SRD has diverse, interacting risk factors at various levels (Miciak & Fletcher, 2020): neurobiological (genetic factors and brain structure and function), cognitive (e.g., phonemic and morphological awareness), behavioral (e.g., attentiveness and motivation), and environmental (e.g., socioeconomic and schooling contexts). The focus of the current study is on the neurobiological level, specifically how genetic and brain factors interact to influence reading ability or disability.

Relationships between brain structure and reading have been clearly established in the field. Most research has focused on cortical structures, with three major reading circuits identified: the dorsal temporo-parietal pathways associated with phonological processing, a ventral occipito-temporal pathway associated with word identification and automatic word recognition, and an anterior frontal region involved in articulation and higher order reading processes (D'Mello & Gabrieli, 2018; Richlan, 2020). The basal ganglia and cerebellum have also been associated with procedural learning related to reading, as well as articulation (D'Mello & Gabrieli, 2018; Hancock et al., 2017; Ullman et al., 2020).

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The integrative use of imaging and genetics is referred to as imaging genetics. It is a field that attempts to improve understanding of the connections between genes and behavior through the investigation of brain imaging as an intermediate phenotype, which is argued to be closer to the level of the gene (Flint et al., 2014). For example, a study comparing effect sizes between gene-brain associations and gene-behavior demonstrated that imaging studies were generally associated with larger effects (Rose & Donohoe, 2013). While most existing imaging genetics studies have focused on candidate genes that have already been associated with reading disability, imaging genetics may also allow better detection of genes that may have small effects on behavioral phenotypes. By investigating the relationship between genes and imaging endophenotypes, imaging genetics methods may be used to identify additional relevant genes that affect phenotypes through their effects on the brain (Flint et al., 2014).

Therefore, in the current study, we focus on a gene that has not been previously investigated for its relation to reading disability, *Semaphorin 6D* (*SEMA6D*). However, based on its location in dyslexia locus DYX1, on chromosome 15q21 (Schumacher et al., 2007), and other studies of its function and association with various disorders, it is likely to have an impact on reading. First, *SEMA6D* is part of a family of genes coding for proteins that regulate axon guidance. Semaphorins mediate many other functions, including processes such as establishing the identity of neuronal cell processes, synapse formation, axon pruning, and regulation of dendrite development (Alto & Terman, 2018; Leslie et al., 2011). Many of the genes already associated with reading disability have similar functions, in processes such as neuronal migration, cortical morphogenesis, dendritic spinal plasticity, and axon guidance (Guidi et al., 2018; Hannula-Jouppi et al., 2005; Mascheretti et al., 2017). Importantly, neuronal migration and axon guidance have been proposed to lead to small cortical malformations, which can affect left hemispheric neural circuits involved in reading and learning (Galaburda et al., 2006, 1985). A more recent study suggested that focusing on just neuronal migration is limiting, and other processes such as axon growth, synaptic transmission, and ciliary function may affect reading disorders as well (Guidi et al., 2018). These processes, which through their effects on axons can lead to changes in white matter structure, affect the reading network. Children with reading disability tend to exhibit white matter differences in left temporoparietal areas and frontal regions, with involvement of the left arcuate fasciculus and corona radiata (Vandermosten et al., 2012) and have different developmental trajectories, with delayed white matter development in the reading network (Christodoulou et al., 2017; Lebel et al., 2019).

Second, semaphorin genes have been associated with other neurodevelopmental disorders, including autism spectrum disorder (Mosca-Boidron et al., 2016), language disorder (Chen et al., 2017; Ercan-Sencicek et al., 2012), attention deficit hyperactivity disorder (ADHD) (Demontis et al., 2019; Hawi et al., 2018), and schizophrenia (Arion et al., 2010), all of which have underlying genetic and neural risk factors. It is likely that many neurodevelopmental disorders may have some common underlying genetic causal factors, due to pleiotropy (i.e., the phenomenon that genes can influence two or more phenotypic traits). For example, the gene *CNTNAP2*, which has been associated with reading disability (Gu et al., 2018; Peter et al., 2011), has also been implicated in autism spectrum disorder, schizophrenia, intellectual disability, and language impairment (Rodenas-Cuadrado et al., 2014). Language impairment and reading disability share common deficits in underlying cognitive processes, such as phonological processing and language fluency (Pennington & Bishop, 2009), so there is likely overlap in genetic contributions to these disorders. Genome-wide association studies searching for genes associated with SRD have implicated genes that are involved in learning in general (Eicher et al., 2013; Gialluisi et al., 2014; Veerappa et al., 2013), and many genes that were previously associated with SRD have been demonstrated to influence language skills also (Eicher & Gruen, 2015). *SEMA6D* has also been associated with educational attainment (Okbay et al., 2016), indicating that the variation in this gene might be associated with overall learning or cognition, directly or indirectly through reading or other academic functions. All of this is evidence that there are generalist genes that can contribute to multiple related traits (Kovas & Plomin, 2006). Therefore, because *SEMA6D* has been associated with other developmental disorders, including autism spectrum disorder and language disorder, there is a strong possibility it could be involved in SRD as well.

Few imaging genetics studies have focused on *SEMA6D*. However, there is evidence that single nucleotide polymorphisms (SNPs) in *SEMA6D* are associated with brain phenotypes as well, as Klein et al. (2019) demonstrated that *SEMA6D* was related toattention deficit hyperactivity disorder (ADHD) risk, as well as intracranial volume and volume of the putamen of the basal ganglia (Klein et al., 2019). The basal ganglia have been shown to be important in procedural learning, impacting learning of language and reading and underlying many neurodevelopmental disorders including reading

disability (Ullman et al., 2020). Variation in other genes in the semaphorin family with related functions have been shown to affect brain structure as well. For example, specific mutations in *SEMA6A*, also involved in axon guidance, have been found to affect brain cellular organization and connectivity in mice, which investigators reported modeled brain changes in other neurodevelopmental disorders such as autism spectrum disorder and schizophrenia (Rünker et al., 2011). Alterations in expression of the *SEMA* gene family have been linked to structural changes in the prefrontal cortex and synapse function associated with schizophrenia (Arion et al., 2010). Further research needs to be conducted to determine whether *SEMA6D* may have effects on other brain imaging phenotypes and how this may relate to reading.

The aim of the current study was to investigate the association between variants in *SEMA6D* and reading-related brain and behavior phenotypes. Specifically, associations between SNPs in or close to *SEMA6D* with various imaging phenotypes (cortical thickness, gyrification, and white matter volume) in reading-related regions of interest in the brain were explored. In turn, relationships between brain structure and measures of word reading fluency and phonological processing were investigated.

Method

Participants

Data were collected from two sites: University of California, San Francisco (UCSF), CA, and Haskins Laboratories in New Haven, CT. These studies were approved by the Yale University Institutional Review Board (Original IRB #1208010711, Re-analysis IRB # 2000021826) and the Stanford University Institutional Review Board (Original IRB #96574 Re-analysis University of Connecticut IRB#H18-200). Written informed consent was obtained from the parent or legal guardian of minor participants, and assent was obtained from participants age 8 years and older. Due to significant variability across scanner strengths and other parameters and the relevance of this variability to the analyses of gyrification and cortical thickness (Han et al., 2006), the data from the two sites could not be merged. Because of the differences in sample size, data collected from Haskins laboratories were used as the primary sample, and data collected from UCSF were used as a replication sample. For volume measures of regions of interest, which tend to be more comparable across different scanner strengths (McCarthy et al., 2015), the samples were combined, and site was used as a covariate in the analysis. Inclusion criteria for both samples required native English language, an IQ above 75, no history of severe developmental or neuropsychological disorders, normal or corrected to normal vision, and normal hearing. Participants from both samples had a broad range of reading abilities. The sample size for the Haskins Laboratories data was 67, collected between 2006 and 2012, which was a subset of participants with both imaging and genetic data from within a larger data set. The age range of the participants was 6–13 years (mean = 9.07), and all participants were Caucasian (white) within the subset used in the current study. The sample size for the UCSF data was 28, collected between 2008 and 2012. The age range of these participants was 5–8 years (mean = 6.50). Race/ethnicity data for the UCSF sample was 63% White/Caucasian, 4% Asian, 7% American Indian/Alaskan Native, 11% multiracial, and 15% unreported.

Behavioral assessments

Assessments of word reading included the Test of Word Reading Efficiency (TOWRE; Torgesen et al., 1999), a timed measure of an individual's ability to read printed words (TOWRE: sight word efficiency) and pseudowords (TOWRE: phonemic decoding efficiency) accurately and fluently. The Comprehensive Test of Phonological Processing (CTOPP; Wagner et al., 1999) elision subtest was used to assess phonological awareness and processing. These measures were used at both sites. For all reading measures, analyses were done with raw scores.

Genetic data

Oragene saliva kits (DNA Genotek, Inc.) were used to obtain saliva samples during behavioral testing sessions and DNA was extracted from the samples according to manufacturer's protocol. DNA libraries were prepared for microarray genotyping on Illumina's HumanCoreExome v1 panel according to the manufacturer's protocol, and genotyping was carried out by Illumina, Inc., San Diego, CA, U.S.A. Illumina's GenomeStudio for Windows software was used for allele calling. Following quality assurance procedures, call rates were evaluated. All samples had a call rate (the fraction of called SNPs per sample over the total number of SNPs in the data set) above 95%, and SNP markers with a call rate below 95% were excluded from the data set. For the UCSF data, samples were collected using peripheral blood. Genotyping was done using the Illumina Core Exome v. 1.2 according to the manufacturer's protocol.

Overall, 67 SNPs in *SEMA6D* were common to data collected at UCSF and at Haskins laboratories. SNPs were analyzed for linkage disequilibrium (Figure 1) and those in high linkage disequilibrium ($R^2 > 0.90$) were removed, leaving 55 SNPs remaining. Because of the small sample size, genotype was coded by the

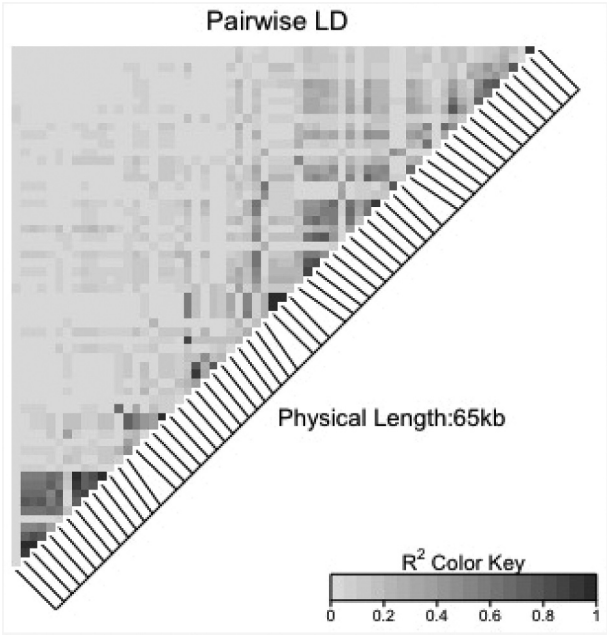


Figure 1. Linkage disequilibrium for *SEMA6D* SNPs.

presence of the derived, or nonancestral, allele (0 without the presence of the derived allele, and 1 with the presence of the derived allele). The ancestral allele is the allelic state of the last common ancestor, while the derived allele is the one that arose due to mutation.

Imaging data

T2 structural magnetic resonance imaging (MRI) data were analyzed in order to obtain data on cortical thickness, gyrification, and white matter volume. These data were collected on two different MRI scanners. Acquisition of brain images by Haskins Laboratories was conducted using a Siemens Sonata 1.5-Tesla MRI scanner with a 12-channel head coil. High-resolution anatomical images were acquired (sagittal Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) acquisition, FA 8°; echo time (TE) 3.65 ms; repetition time (TR) 2000 ms; field of view (FOV) 256 × 256 mm; 1 mm slice thickness, no gap; 256 × 256 × 160, 1 number of excitations (NEX)). Acquisition at UCSF was conducted using a 3T GE Signa scanner with an eight-channel head coil (FSPGR3D-1nex Acquisition; FA 15°; TE 3.4 ms; TR 8.5 ms; FOV 220 × 220 mm; 1 mm slice thickness, no gap; 256 × 192 × 128 matrix, 1 NEX).

MRI preprocessing was conducted to prepare MRI data for analysis using Freesurfer v. 6.0.0 software (Dale et al., 1999; Fischl et al., 1999). The preprocessing pipeline involves reconstruction of a two-dimensional cortical surface into a three-dimensional volume, skull stripping, classification of white and gray matter, and correction for motion. Manual visual inspection and preprocessing was done to correct errors in the pial boundary (between the gray matter and the skull) and the white matter surface boundary, and to correct intensity normalization errors. Cortical thickness was calculated as the shortest path between vertices on pial and white matter boundaries. Spatial smoothing was conducted using a 10-mm full width at half maximum (FWHM) Gaussian filter. A local gyrification index (LGI), measuring the amount of folding in the brain, was calculated using Freesurfer as well; specifically, the LGI quantifies the amount of cortex buried within the sulcal folds as compared to the amount on the outer cortex (Schaer et al., 2012). For the gyrification analysis, spatial smoothing was conducted using only a 5-mm FWHM Gaussian filter because smoothing was already done as part of the automated calculation of the LGI, with gyrification estimations based on 15-mm diameter spheres.

Regions of interest (ROIs) were isolated using preexisting labels of the Destrieux Atlas (Destrieux et al., 2010). These regions of interest were chosen based on their previous association with SRD (Ma et al., 2015), which included the left hemisphere inferior frontal gyrus (pars opercularis, pars orbitalis, and pars triangularis), superior temporal gyrus, Heschl's gyrus, inferior occipital gyrus, planum polare and planum temporale, fusiform gyrus, supramarginal gyrus, and angular gyrus. Similar to the methods used by Ma et al. (2015), a cortical mask was created over the ROIs, and vertex-based analysis of cortical thickness and gyrification was conducted within the mask. The white matter volume associated with each gray matter ROI was extracted from each subject, and analyses were conducted in Freesurfer software with the Killiany/Desikan parcellation atlas (Desikan et al., 2006). For white matter volume, regions of interest were the white matter underlying the supramarginal gyrus, pars opercularis, pars triangularis, pars orbitalis, fusiform gyrus, transverse temporal gyrus, the superior temporal gyrus, and the left and right cerebellum. The names of the ROIs included are based on the cortex because of the way Freesurfer labels white matter, but the regions of interest were actually the underlying white matter associated with these gray matter ROIs.

Statistical analyses

Statistical analyses were completed first for gyrification and cortical thickness phenotypes, then for white matter volume. For cortical thickness and gyrification phenotypes, the steps were as follows: (1)

a vertex-based analysis for genetic associations with imaging phenotypes was done within a mask over all relevant ROIs; (2) significant clusters were extracted and associations with reading were examined; (3) significant SNPs were regressed on reading phenotypes; and (4) a haplotype analysis was completed combining the effects of all SNPs within *SEMA6D*, which were regressed on significant clusters of gyrification or cortical thickness. Following these analyses, significant clusters were not found to be related to reading. Therefore, for white matter volume analyses, for which the measure of white matter volume was calculated over the whole ROI, the analysis was done first to determine brain regions associated with reading. This helped to focus the analysis on the ROIs that were relevant to reading in this sample and increase power to detect genetic effects by reducing the number of imaging genetic analyses. Therefore, for part 2 of the analysis, focused on white matter volume, the steps were as follows: (1) white matter volume was regressed on reading measures; (2) white matter volume of regions significantly related to reading was associated with SNPs; (3) significant SNPs were regressed on reading phenotypes; and (4) a haplotype analysis was completed with white matter volume in the same brain regions, but combining effects of all SNPs within *SEMA6D*. These steps are described in further detail below.

Part 1: Gyrification and cortical thickness analyses

Because the data were collected across two sites that differ on a number of factors (including scanner strength: USCF = 3 T and Haskins = 1.5 T), we elected to first analyze data from Haskin's Laboratories (1.5 T scanner, n = 67), and then replicate the analysis with the UCSF sample (3 T scanner, n = 28). This was done for both cortical thickness and gyrification analyses. A multiple regression analysis was conducted to analyze the associations between SNPs in *SEMA6D* and cortical thickness and gyrification in the brain. After accounting for linkage disequilibrium and removing 12 SNPs, each of the remaining 55 SNPs was used in a separate analysis. All analyses included age and sex as covariates. Correction for multiple comparisons accounting for spatial correlation was done using a Monte Carlo simulation on Freesurfer software with a cluster-forming p value set at $p < .01$, and clusterwise p value set at .05, to reduce false positive rates with cluster-wise corrections. Cortical thickness or gyrification means from significant clusters with any significant SNPs were then used as predictors in a multiple regression analysis to determine whether the LGI and cortical thickness in significant clusters predicted reading. Following analyses of individual SNPs, significant clusters identified in individual SNP analyses were used in a haplotype analysis to determine effects of the entire gene within the Haskins sample. The haplo.stats package in R (Sinnwell & Schaid, 2016) was used to quantify effects of all SNPs in *SEMA6D*, taking into account the fact that SNPs tend to be inherited together, using the haplo.glm function. Haplotype analyses were adjusted for age and gender.

Part 2: White matter volume analyses

For white matter volume, analyses were done across the entire sample because broader measures of volume in ROIs have been found to be more comparable across different scanner strengths (McCarthy et al., 2015). For these analyses, data collection site was included as a covariate. The Shapiro–Wilk test (Shapiro & Wilk, 1965) was carried out to determine normality, and non-normal variables were scaled for further analysis. Partial correlations were first completed to examine whether white matter volume in regions of interest predicted performance on reading measures, controlling for age, gender, and data collection site. Brain associations with reading were examined first to focus this analysis on the brain regions known to be involved in reading. The regions that predicted reading were then further analyzed to determine genetic associations, using multiple regression, controlling for age, gender, and site.

Whole brain volume was not controlled for in these regressions because whole brain volume was not significantly correlated with white matter volume in any of the ROIs investigated or any of the reading measures. Each SNP was used in a separate multiple regression for each brain region, and then, for the analysis of each SNP, correction for multiple comparisons of brain regions was done using the false discovery rate (FDR; Benjamini & Hochberg, 1995). FDR correction for multiple comparisons was used rather than the Monte Carlo cluster-wise correction for multiple comparisons because the analysis was done with average volume over ROIs rather than a vertex-wise analysis. Lastly, multiple regressions were used to analyze associations between SNPs and reading measures, controlling for age, gender, and site, using FDR correction for multiple comparisons. The FDR correction for multiple comparisons results in a q-value, or the expected proportion of false positives among all positive results. Following analyses of individual SNPs, the white matter volume in the same brain regions was used in a haplotype analysis to determine effects of the whole gene, using the haplo.glm function in the haplo.stats R package. Within the haplo.stats R package, the most common

Table 1. Demographic and reading descriptive statistics.

Variable	Haskins (n = 67) *M(SD)*	UCSF (n = 28) *M(SD)*	t-test
Years at age of MRI	9.07 (range 6–13)	6.5 (range 5–8)	t = −11.74, p < 2.2e−16
Sex (% male)	64	55	χ^2 = 89, p = 0.33
IQ measure	WASI 111.68 (13.12)	WJ BIA 118.90 (10.97)	t = 2.78, p = .007
CTOPP elision raw scores	13.48 (4.98)	10.17 (678)	t = −1.09, p = 0.04
TOWRE sight word raw scores	57.85 (18.48)	30.17 (27.10)	t = −4.09, p = 9.74e−05
TOWRE phonemic decoding raw scores	27.30 (13.26)	12.97 (12.34)	t = −3.16, p = 0.0022

Abbreviations: MRI: magnetic resonance imaging; IQ: intelligence quotient; WASI: Wechsler Abbreviated Scale of Intelligence; WJ BIA: Woodcock Johnson Brief Intellectual Ability; CTOPP: Comprehensive Test of Phonological Processing; TOWRE: Test of Word Reading Efficiency.

haplotypes (frequency of greater than .05) are each analyzed separately for effects on phenotypes, while rare haplotypes are combined and analyzed together. Analyses were adjusted for age, gender, and site.

Results

Behavioral results

Demographic and behavioral data are presented in Table 1. The Haskins sample was overall older than the UCSF sample. Intelligence tended to be within the average range as well, but differed significantly across groups, with the UCSF sample tending to have a higher IQ. On reading measures, raw scores varied due to the differing age ranges of the two samples.

SEMA6D-cortical thickness analyses

To assess associations between *SEMA6D* and cortical thickness in the reading network, multiple regression analyses were done for each SNP using age and gender as a covariate, first in the Haskins sample, followed by the UCSF sample. These analyses were done across the reading network using a mask. Correction for multiple comparisons within the brain were done with a Monte Carlo simulation. In the Haskins sample, one SNP (rs16959669) demonstrated a significant association with cortical thickness in the fusiform gyrus after correcting for cluster-wise comparisons (Figure 2). Specifically, the presence of the nonancestral (derived) allele (denoted by 2; n = 5) was associated with greater cortical thickness as compared to SNPs that were homozygous for the ancestral allele (n = 58). This SNP, which has a sample frequency of 0.06 for the derived allele, has been previously shown to be related to skin pigmentation, but has not been previously investigated for relation to reading.

Results of haplotype analyses combining effects from all SNPs in *SEMA6D* revealed significant effects of one haplotype (t = 2.55, p = 0.014) on cortical thickness in the fusiform gyrus cluster. The haplotype frequency was 0.024 and it included the following alleles for each SNP (2 indicating the derived allele), in order by position

Cluster	Maximum p-value	Peak Vertex (Vertex number at which maximum p-value)	Size (mm^2)	MNI Coordinates	Cluster-wise p-value
1	0.0002	28473	706.26	(-41 -67, -13)	0.0016

Figure 2. Significant cortical thickness cluster in fusiform gyrus for rs16959669, with the presence of the non-ancestral allele associated with greater cortical thickness. Peak vertex describes the location of the vertex where the maximum effect was identified for the cluster. Abbreviation: MNI: Montreal Neurological Institute.

in the gene: 21111111111111112111112112211111111-11111121111111211122211. Other haplotypes were not significantly related to cortical thickness.

When replication analyses were performed in the UCSF sample, using the same SNP (rs16959669; ancestral allele n = 22; presence of derived allele n = 5) there was a small cluster nearby (in the lateral occipital region) with an uncorrected significance at $p < .01$, along with other small clusters throughout the reading network (pars opercularis, supramarginal gyrus, and the superior temporal region), but none were significant following cluster-wise correction for multiple comparisons.

Cortical thickness and reading

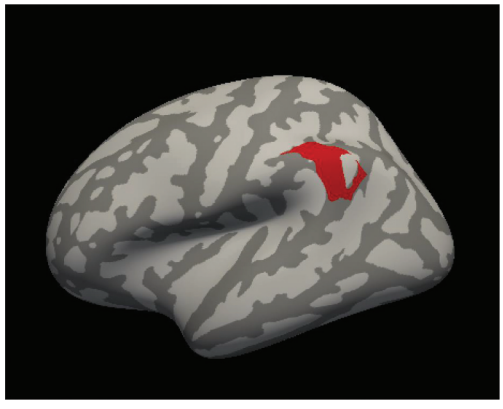
A multiple regression analysis, controlling for age and gender, was completed to assess the association between thickness in the significant cluster and reading in the Haskins sample in separate regression analyses for each reading phenotype. Results of the multiple regression revealed no significant associations between thickness in this cluster and measures of phonological processing (CTOPP elision), word reading (TOWRE sight word efficiency), or nonword reading (TOWRE phonemic decoding).

The association between the SNP rs16959669 and reading was examined in a separate regression, but it was not found to be significantly related to the CTOPP elision raw score ($p = 0.26$), the TOWRE sight word efficiency ($p = 0.56$), or the TOWRE phonemic decoding efficiency ($p = 0.47$).

SEMA6D-gyrification analyses

Multiple regressions, controlling for age and gender, were carried out first in the Haskins sample, followed by the UCSF sample, to determine genetic associations with local gyrification in the reading network (specified by a mask over all of the included ROIs). In the Haskins sample, one SNP, rs4270119 (ancestral allele n = 25; presence of derived allele n = 38), was significantly associated with local gyrification in the supramarginal gyrus after correction for cluster-wise multiple comparisons (Figure 3). Specifically, the presence of the ancestral allele was associated with a smaller LGI. This SNP, rs4270119, has not previously been studied in the literature. The general population frequency of the derived allele is 0.30.

Two other SNPs (rs1369645 [ancestral allele n = 25; presence of derived allele n = 38; general population frequency of derived allele 0.32] and rs16952896 [ancestral allele n = 25; presence of derived allele n = 38; general population frequency of derived allele 0.30]) had very similar significant clusters in the supramarginal gyrus at $p < .01$, but these did not survive correction for multiple comparisons. Results of haplotype analyses combining effects from all SNPs in *SEMA6D* revealed significant effects of two haplotypes (haplotype 1 [1111111 11111111111112112111212222122-22111221112112221 111]: $t = -3.13$, $p = 0.003$, frequency = 0.073; haplotype 2 [222212221212222-1122211121112122221222211122111 2112221111]: $t = -2.88$, $p = 0.006$, frequency = 0.040) on gyrification in the supramarginal gyrus cluster. Other common haplotypes were not significantly associated with gyrification.



Cluster	Maximum p-value	Peak Vertex (Vertex number at which maximum p-value)	Size (mm^2)	MNI Coordinates	Cluster-wise p-value
1	0.00027	5031	878.68	(-59, -47, 33)	0.027

Figure 3. Significant cluster of gyrification in supramarginal gyrus associated with rs4270119. Abbreviation: MNI: Montreal Neurological Institute.

When replication analyses were performed in the UCSF sample, using the same SNPs (rs4270119 [ancestral allele n = 12; presence of derived allele n = 15], rs1369645 [ancestral allele n = 13; presence of derived allele n = 14], and rs16952896 [ancestral allele n = 11; presence of derived allele n = 16]), there were no significant clusters following correction for multiple comparisons.

Gyrification and reading

A multiple regression was carried out in the Haskins sample to determine associations between local gyrification values in the significant supramarginal gyrus cluster and reading measures, controlling for age and gender. Results revealed that gyrification in the supramarginal gyrus was not significantly associated with measures of phonological processing (CTOPP elision), word reading (TOWRE sight word efficiency), or nonword reading (TOWRE phonemic decoding).

The significant SNP, rs4270119, was also regressed on reading, controlling for age, gender, and site, but was not significantly related to the CTOPP elision raw score (p = .60), TOWRE sight word efficiency (p = .70), or TOWRE phonemic decoding efficiency (p = 0.87).

White matter volume and reading

White matter volume values in each region of interest were assessed for normality. ROIs that were not normally distributed (pars opercularis and transverse temporal) were scaled, and these scaled variables were used in the following multiple regression analyses. For white matter volume analyses, white matter volume under gray matter ROIs (left hemisphere supramarginal, pars triangularis, pars opercularis, pars orbitalis, transverse temporal, superior temporal, right and left cerebellum) were partially correlated with reading measures, using age, gender, and scanner as covariates and using FDR correction for multiple comparisons of brain regions. The white matter volume of the left transverse temporal gyrus was significantly associated with all reading phenotypes, including the CTOPP elision raw scores (r = 0.38, p = 0.00037, q = 0.0015), TOWRE sight word efficiency (r = 0.24, p = 0.026, q = .026), and TOWRE phonemic decoding (r = 0.24, p = 0.026, q = 0.026).

Other white matter volume ROIs were also significantly related to the CTOPP elision raw scores, but not other reading measures. These included the pars triangularis (r = 0.31, p = 0.005, q = 0.02), the left cerebellum (r = 0.36, p = 0.00075, q = 0.0030), and the right cerebellum (r = 0.34, p = 0.0016, q = 0.0064). Only these four regions were considered for further analyses.

SEMA6D-white matter volume and SNP-reading analyses

Because it had the strongest association with reading, white matter volume of the transverse temporal gyrus was further analyzed for association with SNPs from *SEMA6D* using multiple regressions controlling for gender, age, and scanner. Three SNPs, rs1817178 (ancestral allele n = 53; presence of derived allele n = 38; general population frequency of derived allele 0.21), rs12050859 (ancestral allele n = 77; presence of derived allele n = 12; general population frequency of derived allele 0.07), and rs1898110 (ancestral allele n = 20; presence of derived allele n = 70; general population frequency of derived allele 0.49) were all significantly associated with white matter volume in the transverse temporal region. For rs1817178, presence of the non-ancestral allele was associated with decreased white matter volume. For rs12050859 and rs1898110, the presence of the non-ancestral allele was associated with increased white matter volume in the transverse temporal region. These three SNPs have not been previously studied for phenotypic associations in the literature.

These SNPs were further analyzed for association with other brain regions that were associated with reading (CTOPP elision raw scores), i.e., the pars triangularis, left cerebellum, and right cerebellum. Rs1817178 was significantly related to white matter volume of the transverse temporal region, but not after correction for multiple comparisons (p = 0.03, q = 0.11), and was not related to white matter in the pars triangularis (p = 0.17, q = 0.17), left cerebellum (p = 0.08, q = 0.12), or the right cerebellum (p = 0.09, q = 0.12). Rs12050859 was nominally related to the white matter of the transverse temporal (p = 0.0135, q = 0.054) and left cerebellum (p = 0.033, q = 0.066) after correction for multiple comparisons, but not the pars triangularis (p = 0.35, q = 0.47) or right cerebellum (p = 0.92, q = 0.92). Rs1898110 was significantly related to white matter in the transverse temporal region before correction for multiple comparisons, but was not related to white matter in the transverse temporal (p = 0.046, q = 0.18), pars triangularis (p = 0.82, q = 0.82), left cerebellum (p = 0.17, q = 0.23), or right cerebellum (p = 0.16, q = 0.23) after correction.

Further analysis of these SNPs in a separate model examining genetic associations with reading, controlling for age, gender, and site and using FDR correction for multiple comparisons, revealed that the SNP rs1817178 also predicted reading, including CTOPP elision raw scores (p = 0.004, q = 0.013), TOWRE sight word efficiency raw scores (p = 0.03, q = 0.04), and TOWRE phonemic decoding efficiency (p = 0.05, q = 0.05). Both rs12050859 and rs1898110 were not significantly associated with reading.

Haplotype analyses were completed for white matter volume in each brain region associated with reading in order to determine effects of the whole gene (taking into account the fact that alleles are linked and inherited together), controlling for age, gender, and scanner. Results of the haplotype analysis revealed significant associations of all of the most common haplotypes (with expected counts greater than 5) with all four investigated brain regions. For the transverse temporal gyrus, the most frequent haplotype [11111111111111111111221111112-11111111111111212121111111122], with a frequency of 0.057, was significantly associated with white matter volume ($t = -1.85 \times 10^3$, $p = 0.00$). For the pars triangularis white matter volume, the association with the same haplotype was also significant ($t = -2.20 \times 10^3$, $p = 0.00$). For the left cerebellum white matter volume, the same haplotype also demonstrated a significant association ($t = 4.73 \times 10^{17}$, $p = 0.00$). Finally, the same was observed for the right cerebellum white matter ($t = 1.810 \times 10^{18}$, $p = 0.00$).

Discussion

The current study investigated whether the analyzed SNPs in the *SEMA6D* gene were related to brain structure and reading in a sample of children at various reading levels, using an integrative imaging genetic approach. Overall, we found relationships between SNPs in *SEMA6D* and brain structure indicators of gyrification, cortical thickness, and white matter volume in the reading network. Taking into consideration the whole gene, there were also strongly significant results between the most common haplotypes and white matter volume in reading-related regions, as well as associations between several haplotypes and cortical thickness in a fusiform gyrus cluster and gyrification in a supramarginal gyrus cluster. However, the gyrification and cortical thickness findings, which were obtained from the Haskins sample, were not replicated in the UCSF sample, potentially due to the small sample size in the UCSF sample.

SEMA6D, with its role in axon guidance and synapse formation, likely affects brain structure and function during brain development. When examining gene networks that *SEMA6D* is involved in, *SEMA6D* has been shown to work in tandem with the *PLXN* family of genes, as plexin proteins act as receptors for semaphorin proteins (Alto & Terman, 2018). *PLXN* genes have been associated with dyslexia, dyspraxia, and language impairment (Rudov et al., 2013), as well as autism (Suda et al., 2011). Similarly, *SEMA6D* has been associated with autism (Mosca-Boidron et al., 2016) and language disorder (Chen et al., 2017; Ercan-Sencicek et al., 2012), and, based on our current results, likely has an effect on reading as well. Furthermore, these related *PLXN* genes have been shown to have effects on white matter structure (Belyk et al., 2017), consistent with our results for *SEMA6D* in the current study. Other reading-disability-related genes, including *KIAA0319* and *ROBO1*, are related to axon growth and guidance as well (Franquinho et al., 2017). Therefore, the current results are consistent with expectations based on *SEMA6D*'s functions, gene networks, and effects on related disorders such as language disorder.

Regarding the link between brain structure and reading, the strongest associations were between white matter volume in the left transverse temporal region (Heschl's gyrus) and measures of phonological processing, word reading, and decoding of nonwords. Previous studies have demonstrated the importance of Heschl's gyrus in reading, and particularly phonologically based learning. For example, Welcome and Joanisse (2014) demonstrated that white matter volume in Heschl's gyrus predicted nonword reading skills in adults. In addition to white matter volume, other studies reveal corresponding associations between gray matter volume of Heschl's gyrus and reading. The size of the left hemisphere Heschl's gyrus, along with differences in planum temporale asymmetry and cerebral volume size, have also been shown to help distinguish between children with phonologically based reading disability and children with language impairment, with SRD children having a larger Heschl's gyrus (Leonard et al., 2002).

Furthermore, the pattern of larger Heschl's gyrus predicted phonological decoding skills in typically developing children (Leonard et al., 2002). Similarly, Wong et al. (2008) found that the volume of left Heschl's gyrus was negatively related to ability to learn pitch patterns, important when learning spoken language. An increased white matter volume, indicating more or stronger connections with a gray matter region of interest, corresponding with a smaller gray matter volume, reflective of increased gray matter density and more efficient processing, tends to be associated with improvements in cognition. Therefore, these corresponding findings demonstrate the importance of Heschl's gyrus in reading. Furthermore, cortical thickness studies provide additional evidence, as thicker cortical thickness in relevant brain regions tends to be associated with improved cognition. In a Norwegian sample, children who later developed dyslexia had thinner cortex in the left hemisphere Heschl's gyrus (along with other primary auditory and visual regions) prior to learning how to read (Clark et al., 2014). Similarly, cortical thickness in the left superior temporal cortex, partially overlapping with Heschl's gyrus, has been shown to be positively correlated with word and

pseudoword reading in typically developing children (Perdue et al., 2020). Overall, across phenotypes, corresponding increases in white matter volume, decreases in gray matter volume, and thicker cortex tend to be related to improvements in reading.

Because the children in the current sample are young (some only 5 or 6 years old), and measures that were used involved basic word reading and decoding and phonological processing, the white matter under Heschl's gyrus may have been more important in affecting reading ability, rather than white matter under other structures, such as the fusiform gyrus, that become more important as there is development of fluent reading, automatic recognition of words, and higher order processing of meaning (Devlin et al., 2006). In our study, *SEMA6D* SNPs and haplotypes had effects on several reading-related regions like the supramarginal gyrus and fusiform gyrus, but only the white matter underlying the transverse temporal gyrus was related to reading. Therefore, it is possible that *SEMA6D* may have a different impact in affecting reading as children develop, because the effects of *SEMA6D* on reading-related structures may have a greater impact on reading during different stages. In other words, these regions may have a more important moderating effect on the link between *SEMA6D* and reading as children age and reading becomes more developed. Earlier development of connectivity between regions tends to predict later functions of reading regions of interest (Saygin et al., 2016), suggesting that the role of certain regions of interest that develop later in reading may not be evident at early ages, with early connectivity developing first. During reading development, children demonstrate changing patterns of functional activation, with readers having more involvement in the inferior frontal gyrus, precentral and postcentral gyrus, and fusiform gyrus during reading compared to pre-readers (Chyl et al., 2018), while activation in superior temporal regions related to speech processing is evident in both pre-readers and emergent readers (Chyl et al., 2018). Therefore, using a sample of older children may show differential impacts of *SEMA6D* on reading as functional networks change and develop. This may also help to explain why the results for cortical thickness and gyrification in the Haskins sample were not replicated in the UCSF sample, as the age ranges were variable.

Three SNPs in *SEMA6D* (rs1817178, rs12050859, rs1898110) were significantly associated with white matter volume in the left hemisphere transverse temporal region, and white matter volume in the left transverse temporal region was significantly related to all three measures of phonological processing, word reading, and decoding of nonwords. Of the three SNPs that were significantly related to white matter volume in the left transverse temporal region, rs1817178 was the only one significantly associated with the reading measures when controlling for age, site, and gender. These findings are novel, because these SNPs have not been previously studied for phenotypic associations in the literature. All three of these SNPs were intron variants of *SEMA6D*, which can have effects on gene expression due to regulatory elements. Therefore, these SNPs may influence expression of *SEMA6D*, which can then lead to changes in brain structure or function. Furthermore, results of the haplotype analysis indicated strong associations between the entire gene and white matter volume in all four regions that were related to reading. However, due to the low frequency of each haplotype when considering all available markers in the gene, the effects of haplotypes should be studied in larger samples to better understand these promising preliminary results. Future studies with larger samples would also benefit from an analysis of how these relevant haplotypes are associated with reading scores as well. Because *SEMA6D* has known functions in axon guidance and synapse formation, it likely influences the development of the brain and white matter structure in the brain.

Other findings were that SNPs in *SEMA6D* were significantly associated with gyrification in the left hemisphere supramarginal gyrus and cortical thickness in the left hemisphere fusiform gyrus in the Haskins sample. However, these results were not replicated in the UCSF sample, potentially due to its small sample size. Additionally, gyrification in the supramarginal gyrus and cortical thickness in the fusiform gyrus were not significantly related to reading. However, gyrification and cortical thickness in the reading network have been shown to be related to reading in previous research. For example, Blackmon et al. (2010) demonstrated that better pronunciation of irregular words (more representative of the orthographic components of word recognition) was associated with thinner cortex in reading network regions including the supramarginal gyrus.

Alternatively, in a sample of Chinese children, cortical thickness in the left supramarginal gyrus was positively correlated with oral word reading, and also predicted phonological awareness (Xia et al., 2018). One study looking at both gyrification and cortical thickness found corresponding increased gyrification and thinner cortex in the left occipitotemporal region, where the fusiform gyrus is located, in children with dyslexia (Williams et al., 2018). Gyrification and cortical thickness tend to be negatively related to each other, with greater gyrification and thinner cortex related to more efficient processing (White et al., 2010).

While we did not find these relationships to reading in the current study, this may have been due to the

young age of the participants and less developed reading skills that are likely still relying on phonological processes rather than orthographic recognition during reading. Future studies may benefit from doing analyses to examine whether there is an interaction with this relationship and age. Furthermore, this sample generally consisted of children who were low-average to above-average readers, not meeting criteria for reading disability. Therefore, there may not have been enough variability in the sample to detect significant relationships between cortical thickness and gyrification and reading. In addition, the smaller sample size (i.e., using the UCSF sample alone) may have made it more difficult to detect significant effects.

Future research may benefit from using a sample of children with reading disability. While effects of SNPs tended to be small and sometimes did not survive after correction for multiple comparisons, the study had an overall small sample size and also used a sample of children with a wide range of reading ability. Therefore, results might have been stronger using a sample of children with diagnosed reading disability compared to typically developing children. Research should also consider gene-by-gene or gene-by-environment interactions, as taking interacting effects into account will help us better understand the relationship between genes and brain structure and reading (Gilbert-Diamond & Moore, 2011). Genes often work as part of a pathway or network, so having a full understanding of interacting effects could improve our understanding of the strength of the relationship between genes and phenotypes.

Additionally, while we examined effects on three different phenotypes – gyrification, cortical thickness, and white matter volume – in the current study, future research should expand on these findings to better understand *SEMA6D*'s effect on the brain. There are limitations to measuring global white matter volume underlying gray matter regions of interest because it limits our understanding of the whole white matter pathway. Therefore, effects on white matter could be better understood by using fractional anisotropy or radial or axial diffusivity for a finer look at impact on white matter structure. In addition, the use of functional methodology, such as fMRI, would improve understanding of how *SEMA6D* influences brain activity, and how this may affect reading. Lastly, future research may benefit from examining other subcortical brain regions, such as the basal ganglia, as well as the corpus callosum and brain regions involved in visual processing, as *SEMA6D* is associated with axon guidance during development of the corpus callosum and retinal mapping (Alto & Terman, 2018).

Conclusions

Overall, the results of the current study suggest that the variation in *SEMA6D* is associated with the variation in the brain structure within the reading network. Specifically, SNPs in *SEMA6D* were associated with gyrification in the supramarginal gyrus, cortical thickness in the fusiform gyrus, and white matter volume in the transverse temporal gyrus. With respect to brain-behavior relations, regardless of genotype, white matter volume in the transverse temporal gyrus was most strongly related to reading, possibly due to the young age of the participants and their still-developing reading skills, likely relying mostly on phonological processing. While *SEMA6D* had effects on several reading-related brain regions, these regions fluctuate in their role in reading development depending on the stage of reading, whether it is phonological processing or automatic recognition of words. Therefore, *SEMA6D*, through its effects on various reading-related brain regions, likely indirectly impacts reading at various stages of reading development. *SEMA6D* has known functions in axon guidance and synapse formation, likely influencing the development of the brain, white matter structure, and synaptic connections. The results show that the *SEMA6D* gene and its variation appear to be associated with individual differences in performance on language and reading, and further research should focus on an older population, more phenotypes focused on white matter, and potential gene interactions with other genes and the environment.

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References

Alto, L. T., & Terman, J. R. (2018). Semaphorins and their signaling mechanisms. *Methods in Molecular Biology*, *1493*, 1–25. https://doi.org/10.1007/978-1-4939-6448-2_1

Arion, D., Horvath, S., Lewis, D., & Mirnics, K. (2010). Infragranular gene expression disturbances in the prefrontal cortex in schizophrenia: Signature of altered neural development? *Neurobiology of Disease*, *37*(3), 738–746. https://doi.org/10.1016/j.nbd.2009.12.013

Belyk, M., Kraft, S. J., Brown, S., & Pediatric Imaging, Neurocognition and Genetics Study. (2017). PlexinA polymorphisms mediate the developmental trajectory of human corpus callosum microstructure. *Journal of Human Genetics*, *60*(3), 147–150. https://doi.org/10.1038/jhg.2014.107

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple hypothesis testing. *Journal of the Royal statistical society: series B (Methodological)*, *57*(1), 289–300. Retrieved from http://www.jstor.org/stable/2346101

Blackmon, K., Barr, W. B., Kuzniecky, R., DuBois, J., Carlson, C., Quinn, B. T., Blumberg, M., Halgren, E., Hagler, D. J., Mikhly, M., Devinsky, O., McDonald, C. R., Dale, A. M., & Thesen, T. (2010). Phonetically irregular word pronunciation and cortical thickness in the adult brain. *NeuroImage*, *51*(4), 1453–1458. https://doi.org/10.1016/j.neuroimage.2010.03.028

Chen, X. S., Reader, R. H., Hoischen, A., Veltman, J. A., Simpson, N. H., Francks, C., Newbury, D. F., & Fisher, S. (2017). Next-generation DNA sequencing identifies novel gene variants and pathways involved in specific language impairment. *Scientific Reports*, *7*(1), 46105. https://doi.org/10.1038/srep46105

Christodoulou, J. A., Murtagh, J., Cyr, A., Perrachione, T. K., Chang, P., Halverson, K., Hook, P., Yendiki, A., Ghosh, S., & Gabrieli, J. D. E. (2017). Relation of white-matter microstructure to reading ability and disability in beginning readers. *Neuropsychology*, *31*(5), 508–515. https://doi.org/10.1037/neu0000243

Chyl, K., Kossowski, B., Dębska, A., Łuniewska, M., Banaszkiewicz, A., Żelechowska, A., Frost, S. J., Mencl, W. E., Wypych, M., Marchewka, A., Pugh, K. R., & Jednoróg, K. (2018). Prereader to beginning reader: Changes induced by reading acquisition in print and speech brain networks. *Journal of Child Psychology and Psychiatry*, *59*(1), 76–87. https://doi.org/10.1111/jcpp.12774

Clark, K. A., Helland, T., Specht, K., Narr, K. L., Manis, F. R., Toga, A. W., & Hugdahl, K. (2014). Neuroanatomical precursors of dyslexia identified from pre-reading through to age 11. *Brain*, *137*(12), 3136–3141. https://doi.org/10.1093/brain/awu229

D'Mello, A. M., & Gabrieli, J. D. E. (2018). Cognitive neuroscience of dyslexia. *Language, Speech, and Hearing Services in Schools*, *49*(4), 798–809. https://doi.org/10.1044/2018_LSHSS-DYSLC-18-0020

Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical surface-based analysis: I. Segmentation and surface reconstruction. *Neuroimage*, *9*(2), 179–194. https://doi.org/10.1006/nimg.1998.0395

Demontis, D., Walters, R. K., Martin, J., Mattheisen, M., Als, T. D., Agerbo, E., Baldursson, G., Belliveau, R., Bybjerg-Grauholm, J., Bækvad-Hansen, M., Cerrato, F., Chambert, K., Churchhouse, C., Dumont, A., Eriksson, N., Gandal, M., Goldstein, J. I., Grasby, K. L., Grove, J., … Neale, B. J. (2019). Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. *Nature Genetics*, *51*(1), 63–75. https://doi.org/10.1038/s41588-018-0269-7

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., Buckner, R. L., Dale, A. M., Maguire, R. P., Hyman, B. T., Albert, M. S., & Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage*, *31*(3), 968–980. https://doi.org/10.1016/j.neuroimage.2006.01.021

Destrieux, C., Fischl, B., Dale, A., & Halgren, E. (2010). Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. *NeuroImage*, *53*(1), 1–15. https://doi.org/10.1016/j.neuroimage.2010.06.010

Devlin, J. T., Jamison, H. L., Gonnerman, L. M., & Matthews, P. M. (2006). The role of the posterior fusiform gyrus in reading. *Journal of Cognitive Neuroscience*, *18*(6), 911–922. https://doi.org/10.1162/jocn.2006.18.6.911

Eicher, J. D., & Gruen, J. R. (2015). Language impairment and dyslexia genes influence language skills in children with autism spectrum disorders. *Autism Research*, *8*(2), 229–234. https://doi.org/10.1002/aur.1436

Eicher, J. D., Powers, N. R., Miller, L. L., Akshoomoff, N., Amaral, D. G., Bloss, C. S., Libiger, O., Schork, N. J., Darst, B. F., Casey, B. J., Chang, L., Ernst, T., Frazier, J., Kaufmann, W. E., Keating, B., Kenet, T., Kennedy, D., Mostofsky, S., Murray, S. S., … Gruen, J. R. (2013). Genome-wide association study of shared components of reading disability and language impairment. *Genes, Brain, and Behavior*, *12*(8), 792–801. https://doi.org/10.1111/gbb.12085

Ercan-Sencicek, A., Wright, N. R. D., Sanders, S. J., Oakman, N., Valdes, L., Bakkaloglu, B., Doyle, N., Yrigollen, C. M., Morgan, T. M., & Grigorenko, E. L. (2012). A balanced t (10;15) translocation in a male patient with developmental language disorder. *European Journal of Medical Genetics*, *55*(2), 128–131. https://doi.org/10.1016/j.ejmg.2011.12.005

Fischl, B., Sereno, M. I., & Dale, A. M. (1999). Cortical surface-based analysis: II: Inflation, flattening, and a surface-based coordinate system. *Neuroimage*, *9*(2), 195–207. https://doi.org/10.1006/nimg.1998.0396

Flint, J., Timpson, N., & Munafó, M. (2014). Assessing the utility of intermediate phenotypes for genetic mapping of psychiatric disease. *Trends in Neurosciences*, *37*(12), 733–741. https://doi.org/10.1016/j.tins.2014.08.007

Franquinho, F., Nogueira-Rodrigues, J., Duarte, J. M., Esteves, S. S., Carter-Su, C., Monaco, A. P., Molnár, Z., Velayos-Baeza, A., Brites, P., & Sousa, M. M. (2017). The dyslexia-susceptibility protein *KIAA0319* inhibits axon growth through *SMAD2* signaling. *Cerebral Cortex*, *27*(3), 1732–1747. https://doi.org/10.1093/cercor/bhx023

Galaburda, A. M., LoTurco, J., Ramus, F., Fitch, R. H., & Rosen, G. D. (2006). From genes to behavior in developmental dyslexia. *Nature Neuroscience*, *9*(10), 1213–1217. https://doi.org/10.1038/nn1772

Galaburda, A. M., Sherman, G. F., Rosen, G. D., Aboitiz, F., & Geschwind, N. (1985). Developmental dyslexia: Four consecutive patients with cortical anomalies. *Annals of Neurology*, *18*(2), 222–233. https://doi.org/10.1002/ana.410180210

Gialluisi, A., Newbury, D. F., Wilcutt, E. G., Olson, R. K., DeFries, J. C., Brandler, W. M., Pennington, B. F., Smith, S. D., Scerri, T. S., Simpson, N. H., Luciano, M., Evans, D. M., Bates, T. C., Stein, J. F., Talcott, J. B., Monaco, A. P., Paracchini, S., Francks, C., & Fisher, S. E. (2014). Genome-wide screening for DNA variants associated with reading and language traits. *Genes, Brain and Behavior*, *13*(7), 686–701. https://doi.org/10.1111/gbb.12158

Gilbert-Diamond, D., & Moore, J. H. (2011). Analysis of gene-gene interactions. *Current Protocols in Human Genetics*, *70*(1), 1.14.1–1.14.12. https://doi.org/10.1002/0471142905.hg0114s70

Gu, H., Hou, F., Liu, L., Luo, X., Nkomola, P. D., Xie, X., Li, X., & Song, R. (2018). Genetic variants in the *CNTNAP2* gene are associated with gender differences among dyslexic children in China. *EBioMedicine*, *34*, 165–170. https://doi.org/10.1016/j.ebiom.2018.07.007

Guidi, L. G., Velayos-Baeza, A., Martinez-Garay, I., Monaco, A. P., Paracchini, S., Bishop, D. V. M., & Molnár, Z. (2018). The neuronal migration hypothesis of dyslexia: A critical evaluation 30 years ago. *European Journal of Neuroscience*, *48*(10), 3212–3233. https://doi.org/10.1111/ejn.14149

Han, X., Jovicich, J., Salat, D., van der Kouwe, A., Quinn, B., Czanner, S., Busa, E., Pacheco, J., Albert, M., Killiany, R., Maguire, P., Rosas, D., Makris, N., Dale, A., Dickerson, B., & Fischl, B. (2006). Reliability of MRI-derived measurements of human cerebral cortical thickness: The effects of field strength, scanner upgrade and manufacturer. *NeuroImage*, *32*(1), 180–194. https://doi.org/10.1016/j.neuroimage.2006.02.051

Hancock, R., Richlan, F., & Hoeft, F. (2017). Possible roles for fronto-striatal circuits in reading disorder. *Neuroscience & Biobehavioral Reviews*, *72*, 243–260. https://doi.org/10.1016/j.neubiorev.2016.10.025

Hannula-Jouppi, K., Kaminen-Ahola, N., Taipale, M., Eklund, R., Nopola-Hemmi, J., Kääriäinen, H., & Kere, J. (2005). The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. *PLOS Genetics*, *1*(4), e50. https://doi.org/10.1371/journal.pgen.0010050

Hawi, Z., Yates, H., Pinar, A., Arnatkeviciute, A., Johnson, B., Tong, J., Pugsley, K., Dark, C., Pauper, M., Klein, M., Heussler, H. S., Hiscock, H., Fornito, A., Tiego, J., Finlay, A., Vance, A., Gill, M., Kent, L., & Bellgrove, M. A. (2018). A case-control genome-wide association study of ADHD discovered a novel association with the tenascin R (TNR) gene. *Translational Psychiatry*, *8*(1), 284. https://doi.org/10.1038/s41398-018-0329-x

Hulme, C., & Snowling, M. J. (2016). Reading disorders and dyslexia. *Current Opinion in Pediatrics*, *28*(6), 731–735. https://doi.org/10.1097/MOP.0000000000000411

Klein, M., Walters, R. K., Demontis, D., Stein, J. L., Hibar, D. P., Adams, H. H., Bralten, J., Mota, N. R., Schachar, R., Sonuga-Barke, E., Mattheisen, M., Neale, B. M., Thompson, P. M., Medland, S. E., Børglum, A. D., Faraone, S. V., Arias-Vasquez, A., & Franke, B. (2019). Genetic markers of ADHD-related variations in intracranial volume. *American Journal of Psychiatry*, *176*(3), 228–238. https://doi.org/10.1176/appi.ajp.2018.18020149

Kovas, Y., & Plomin, R. (2006). Generalist genes: Implications for the cognitive sciences. *Trends in Cognitive Sciences*, *10*(5), 198–203. https://doi.org/10.1016/j.tics.2006.03.001

Lebel, C., Benischek, A., Geeraert, B., Holahan, J., Shaywitz, S., Bakhshi, K., & Shaywitz, B. (2019). Developmental trajectories of white matter structure in children with and without reading impairments. *Developmental Cognitive Neuroscience*, *36*, 100633. https://doi.org/10.1016/j.dcn.2019.100633

Leonard, C. M., Lombardino, L. J., Walsh, K., Eckert, M. A., Mockler, J. L., Rowe, L. A., Williams, S., & DeBose, C. B. (2002). Anatomical risk factors that distinguish dyslexia from SLI predict reading skill in normal children. *Journal of Communication Disorders*, *35*(6), 501–531. https://doi.org/10.1016/S0021-9924(02)00120-X

Leslie, J. R., Imai, F., Fukuhara, K., Takegahara, N., Rizvi, T. A., Friedel, R. H., Wang, F., Kumanogoh, A., & Yoshida, Y. (2011). Ectopic myelinating oligodendrocytes in the dorsal spinal cord as a consequence of altered Semaphorin 6D signaling inhibit synapse formation. *Development*, *138*(18), 4085–4095. https://doi.org/10.1242/dev.066076

Ma, Y., Koyama, M. S., Milham, M. P., Castellanos, F. X., Quinn, B. T., Pardoe, H., Wang, X., Kuzniecky, R., Devinsky, O., Thesen, T., & Blackmon, K. (2015). Cortical thickness abnormalities associated with dyslexia, independent of remediation status. *NeuroImage: Clinical*, *7*, 177–186. https://doi.org/10.1016/j.nicl.2014.11.005

Mascheretti, S., De Luca, A., Trezzi, V., Peruzzo, D., Nordio, A., Marino, C., & Arrigoni, F. (2017). Neurogenetics of developmental dyslexia: From genes to behavior through brain neuroimaging and cognitive and sensorial mechanisms. *Translational Psychiatry*, *7*(1), e987. https://doi.org/10.1038/tp.2016.240

McCarthy, C. S., Ramprashad, A., Thompson, C., Botti, J., Coman, I. L., & Kates, W. R. (2015). A comparison of FreeSurfer-generated data with and without manual intervention. *Frontiers in Neuroscience*, *9*, 379. https://doi.org/10.3389/fnins.2015.00379

Miciak, J., & Fletcher, J. M. (2020). The critical role of instructional response for identifying dyslexia and other learning disabilities. *Journal of Learning Disabilities*, *53*(5), 343–353. https://doi.org/10.1177/0022219420906801

Mosca-Boidron, A., Gueneau, L., Huguet, G., Goldenberg, A., Henry, C., Gigot, N., Pallesi-Pocachard, E., Falace, A., Duplomb, L., Thevenon, J., Duffourd, Y., ST-Onge, J., Chambon, P., Rivière, J.-B., Thauvin-Robinet, C., Callier, P., Marle, N., Payet, M., Ragon, C., … Bourgeron, T. (2016). A *de novo* microdeletion of *SEMA5A* in a boy with autism spectrum disorder and intellectual disability. *European Journal of Human Genetics*, *24*(6), 838–843. https://doi.org/10.1038/ejhg.2015.211

Okbay, A., Beauchamp, J. P., Fontana, M. A., Lee, J. L., Pers, T. H., Rietveld, C. A., Turley, P., Chen, G., Emilsson, V., Meddens, S. F. W., Oskarsson, S., Pickrell, J. K., Thom, K., Timshel, P., de Vlaming, R., Abdellaoui, A., Ahluwalia, T. S., Bacelis, J., Baumbach, C., … Benjamin, D. J. (2016). Genome-wide association study identifies 74 loci associated with educational attainment. *Nature*, *533*(7604), 539–542. https://doi.org/10.1038/nature17671

Pennington, B., & Bishop, D. (2009). Relations among speech, language, and reading disorders. *Annual Review of Psychology*, *60*(1), 283–306. https://doi.org/10.1146/annurev.psych.60.110707.163548

Perdue, M., Mednick, J., Pugh, K. R., & Landi, N. (2020). Gray matter structure is associated with reading skill in typically developing young readers. *Cerebral Cortex*, *30*(10), 5449–5459. https://doi.org/10.1093/cercor/bhaa126

Peter, B., Raskind, W. H., Matsushita, M., Lisowski, M., Vu, T., Berninger, V. W., Wijsman, E. M., & Brkanac, Z. (2011). Replication of *CNTNAP2* association with nonword repetition and support for *FOXP2* association with timed reading and motor activities in a dyslexia family sample. *Journal of Neurodevelopmental Disorders*, *3*(1), 39–49. https://doi.org/10.1007/s11689-010-9065-0

Richlan, F. (2020). The functional neuroanatomy of developmental dyslexia across languages and writing systems. *Frontiers in Psychology*, *11*, 155. https://doi.org/10.3389/fpsyg.2020.00155

Rodenas-Cuadrado, P., Ho, J., & Vernes, S. C. (2014). Shining a light on *CNTNAP2*: Complex functions to complex disorders. *European Journal of Human Genetics*, *22*(2), 171–178. https://doi.org/10.1038/ejhg.2013.100

Rose, E. J., & Donohoe, G. (2013). Brain vs behavior: An effect size comparison of neuroimaging and cognitive studies of genetic risk for schizophrenia. *Schizophrenia Bulletin*, *39*(3), 518–526. https://doi.org/10.1093/schbul/sbs056

Rudov, A., Rocchi, M. B. L., Accorsi, A., Spada, G., Procopio, A. D., Olivieri, F., Rippo, M. R., & Albertini, M. C. (2013). Putative miRNAs for the diagnosis of dyslexia, dyspraxia, and specific language impairment. *Epigenetics*, *8*(10), 1023–1029. https://doi.org/10.4161/epi.26026

Rünker, A. E., O'Tuathaigh, C., Dunleavy, M., Morris, D. W., Little, G. E., Corvin, A. P., Gill, M., Henshall, D. C., Waddington, J. L., & Mitchell, K. J. (2011). Mutation of *Semaphorin-6A* disrupts limbic and cortical connectivity and models neurodevelopmental psychopathology. *PLoS One*, *6*(11), e26488. https://doi.org/10.1371/journal.pone.0026488

Saygin, Z. M., Osher, D. E., Norton, E. S., Youssoufian, D. A., Beach, S. D., Feather, J., Gaab, N., Gabrieli, J. D. E., & Kanwisher, N. (2016). Connectivity precedes function in the development of the visual word form area. *Nature Neuroscience*, *19*(9), 1250–1255. https://doi.org/10.1038/nn.4354

Schaer, M., Cuadra, M. B., Schmansky, N., Fischl, B., Thiran, J., & Eliez, S. (2012). How to measure cortical folding from MR images: A step-by-step tutorial to compute local gyrification index. *Journal of Visualized Experiments*, *59*, e3417. https://doi.org/10.3791/3417

Schumacher, J., Hoffmann, P., Schmäl, C., Schulte-Körne, G., & Nöthen, M. M. (2007). Genetics of dyslexia: The evolving landscape. *Journal of Medical Genetics*, *44*(5), 289–297. https://doi.org/10.1136/jmg.2006.046516

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, *52*(3–4), 482–483. https://doi.org/10.1093/biomet/52.3-4.591

Sinnwell, J. P., & Schaid, D. J. (2016). *Haplo Stats (version 1.7.7) statistical methods for haplotypes when linkage phase is ambiguous*. R package version 1.8.6.

Suda, S., Iwata, K., Shimmura, C., Kameno, Y., Anitha, A., Thanseem, I., Nakamura, K., Matsuzaki, H., Tsuchiya, K. J., Sugihara, G., Iwata, Y., Suzuki, K., Koizumi, K., Higashida, H., Takei, N., & Mori, N. (2011). Decreased expression of axon-guidance receptors in the anterior cingulate cortex in autism. *Molecular Autism*, *2*(1), 14. https://doi.org/10.1186/2040-2392-2-14

Torgesen, J. K., Wagner, R. K., & Rashotte, C. A. (1999). *Test of Word Reading Efficiency (TOWRE)*. Pro-ed.

Ullman, M. T., Earle, F. S., Walenski, M., & Janacsek, K. (2020). The neurocognition of developmental disorders of language. *Annual Review of Psychology*, *71*(1), 389–417. https://doi.org/10.1146/annurev-psych-122216-011555

Vandermosten, M., Boets, B., Wouters, J., & Ghesquière, P. (2012). A qualitative and quantitative review of diffusion tensor imaging studies in reading and dyslexia. *Neuroscience & Biobehavioral Reviews*, *36*(6), 1532–1552. https://doi.org/10.1016/j.neubiorev.2012.04.002

Veerappa, A. M., Saldanha, M., Padakannaya, P., & Ramachandra, N. B. (2013). Family-based genome-wide copy scan number scan identifies five new genes of dyslexia involved in dendritic spinal plasticity. *Journal of Human Genetics*, *58*(8), 539–547. https://doi.org/10.1038/jhg.2013.47

Wagner, R. K., Torgesen, J. K., & Rashotte, C. A. (1999). *Comprehensive test of phonological processing*. Pro-Ed.

Welcome, S. E., & Joanisse, M. F. (2014). Individual differences in white matter anatomy predict dissociable components of reading skill in adults. *NeuroImage*, *96*, 261–275. https://doi.org/10.1016/j.neuroimage.2014.03.069

White, T., Su, S., Schmidt, M., Kao, C., & Shapiro, G. (2010). The development of gyrification in childhood and adolescence. *Brain and Cognition*, *72*(1), 36–45. https://doi.org/10.1016/j.bandc.2009.10.009

Williams, V. J., Juranek, J., Cirino, P., & Fletcher, J. M. (2018). Cortical thickness and local gyrification in children with developmental dyslexia. *Cerebral Cortex*, *28*(3), 963–973. https://doi.org/10.1093/cercor/bhx001

Wong, P. C. M., Warrier, C. M., Penhune, V. B., Roy, A. K., Sadehh, A., Parrish, T. B., & Zatorre, R. J. (2008). Volume of left Heschl's gyrus and linguistic pitch learning. *Cerebral Cortex*, *18*(4), 828–836. https://doi.org/10.1093/cercor/bhm115

Xia, Z., Zhang, L., Hoeft, F., Gu, B., Gong, G., & Shu, H. (2018). Neural correlates of oral word reading, silent reading comprehension, and cognitive subcomponents. *International Journal of Behavioral Development*, *42*(3), 342–356. https://doi.org/10.1177/0165025417727872