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Variants in the *DYX2* locus are associated with altered brain activation in reading-related brain regions in subjects with reading disability

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ABSTRACT

Reading disability (RD) is a complex genetic disorder with unknown etiology. Genes on chromosome 6p22, including *DCDC2*, *KIAA0319*, and *TTRAP*, have been identified as RD associated genes. Imaging studies have shown both functional and structural differences between brains of individuals with and without RD. There are limited association studies performed between RD genes, specifically genes on 6p22, and regional brain activation during reading tasks. Using fourteen variants in *DCDC2*, *KIAA0319*, and *TTRAP* and exhaustive reading measures, we first tested for association with reading performance in 82 parent-offspring families (326 individuals). Next, we determined the association of these variants with activation of sixteen brain regions of interest during four functional magnetic resonance imaging-reading tasks. We nominally replicated associations between reading performance and variants of *DCDC2* and *KIAA0319*. Furthermore, we observed a number of associations with brain activation patterns during imaging-reading tasks with all three genes. The strongest association occurred between activation of the left anterior inferior parietal lobe and complex tandem repeat BV677278 in *DCDC2* (uncorrected $p = 0.00003$, $q = 0.0442$). Our results show that activation patterns across regions of interest in the brain are influenced by variants in the *DYX2* locus. The combination of genetic and functional imaging data show a link between genes and brain functioning during reading tasks in subjects with RD. This study highlights the many advantages of imaging data as an endophenotype for discerning genetic risk factors for RD and other communication disorders and underscores the importance of integrating neurocognitive, imaging, and genetic data in future investigations.

Introduction

Reading disability (RD) or dyslexia is the most commonly observed learning disability with a prevalence in Western countries estimated to be between 5% and 17% (Interagency Committee on Learning Disabilities, 1987; Pennington and Bishop, 2009; Shaywitz et al., 1994). RD is marked by difficulty reading despite adequate education, motivation, and intelligence (Pennington and Bishop, 2009; Shaywitz et al., 1994). Twin and family studies showed that RD has both environmental and genetic determinants with heritability estimates of 44–75% (DeFries et al., 1987). Our group and others have identified various RD associated genes in the *DYX2* locus on chromosome 6p22 including replicated associations of *DCDC2*, *KIAA0319*, and a haplotype including *KIAA0319/TTRAP* (Cope et al., 2005; Francks et al., 2004; Harold et al., 2006; Lind et al., 2010; Luciano et al., 2007; Ludwig et al., 2008; Marino et al., 2012; Meng et al., 2005; Paracchini et al., 2008; Scerri et al., 2011; Schumacher et al., 2006; Wilcke et al., 2009). How these genes function in neuronal circuitry and mechanistically contribute to RD remains unknown, especially since most of the associated variants have not yet been shown to be functional. However, two possible functional variants on *DYX2* have been identified. Our group has identified a microdeletion/complex tandem repeat in

Abbreviations: RD, reading disability; fMRI, functional magnetic resonance imaging; ROIs, regions of interest; GORT, Gray Oral Reading Test; WASI, Wechsler Abbreviated Scale of Intelligence; TOWRE, Test of Word Reading Efficiency; CTOPP, Comprehensive Test of Phonological Processing; WR, Word Rhyming; NWR, Non-Word Rhyming; PC, Print Categorization; AC, Auditory Categorization; PCA, principle component analysis; FDR, false discovery rate; LD, linkage disequilibrium; SAC, superior anterior cingulated gyrus; PC, posterior cingulated gyrus; LPC, left paracentral lobule; LIFGI, left inferior frontal gyrus, inferior aspect; RAIPL, right anterior inferior parietal lobe; LAIPL, left anterior inferior parietal lobe; RLOTG, right lateral occipital termporal gyrus.

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intron 2 of *DCDC2* (GenBank ID: BV677278) that associates with RD, influences *DCDC2* gene expression, and binds a putative transcriptional regulatory complex (Marino et al., 2012; Meng et al., 2005, 2011; Wilcke et al., 2009). Paracchini et al. showed that the *KIAA0319/TTRAP* risk haplotype yielded decreased expression of *KIAA0319* but not *TTRAP* (Paracchini et al., 2006). Additionally, animal studies demonstrated that *DCDC2* and *KIAA0319* are involved in neuronal migration (Levecque et al., 2009; Paracchini et al., 2006; Velayos-Baeza et al., 2008). The role of *DCDC2* and *KIAA0319* in neuronal migration suggests variants of these genes may cause abnormalities in brain morphology and functioning.

Functional magnetic resonance imaging (fMRI) studies show that subjects with RD display different patterns of brain activation during reading tasks compared to non-impaired controls (Brunswick et al., 1999; Horwitz et al., 1998; Paulesu et al., 1996, 2001; Rumsey et al., 1992, 1997; Shaywitz et al., 1998). Subjects with RD also have structural brain differences compared to non-impaired controls, both in grey matter density and in white matter microstructure connectivity (Beaulieu et al., 2005; Brambati et al., 2004; Brown et al., 2001; Deutsch et al., 2005; Eliez et al., 2000; Hoeft et al., 2007; Klingberg et al., 2000; Niogi and McCandliss, 2006; Paulesu et al., 2001; Silani et al., 2005; Vinckenbosch et al., 2005). These functional and structural differences are most prominent in the superior temporal gyrus, inferior frontal gyrus, parietal-temporal gyrus, and left inferior parietal lobule. In general, subjects with RD utilize different brain regions and neuronal circuits during reading tasks compared to non-impaired controls, suggesting determinants of RD alter brain development and functioning.

Despite the evidence of functional and structural differences between individuals with RD and non-impaired controls, the use of imaging data as an endophenotype to explore mechanisms of neurobehavioral disorders remains under-utilized. Specifically, investigations of the interactions among RD associated genes, behavioral measures, and imaging data are few in number and limited in functional imaging data. One study found a relationship between grey matter volume in subjects with RD and the BV677278 microdeletion (Meda et al., 2008). Recent studies by Wilcke et al. in subjects with RD and Pinel et al. in non-impaired individuals found differences among variants in *FOXP2* in regional brain activation during reading tasks including temporo-parietal, inferior frontal, and precentral brain regions (Pinel et al., 2012; Wilcke et al., 2011). Pinel et al. also observed an association between *KIAA0319/TTRAP/THEM2* and lower asymmetric activation of the posterior superior temporal sulcus during reading and phonology tasks. These studies suggest that functional brain patterning can be an informative endophenotype for conditioning genetic associations.

The goals of this investigation are to replicate associations of *DCDC2*, *KIAA0319*, and *TTRAP* with behavioral reading measures and to use fMRI to interrogate brain regions involved in reading tasks in the same subjects. This investigation is one of the first comprehensive analyses of behavioral, functional imaging, and genetic data in the same RD cohort. By using both behavioral and imaging-reading tasks, we aim to strengthen associations of *DYX2* variants with RD and to evaluate functional consequences of RD associated variants using fMRI data. We hypothesize that associations of *DYX2* variants with RD using behavioral data will be replicated and that these variants will influence brain activation patterns during imaging-reading tasks in reading-related regions of interest (ROIs).

Materials and Methods

Subjects

Eighty-two unrelated subjects of European American ancestry (50 RD cases / 25 non-impaired controls / 7 with unknown affectation status) from the Yale Center for the Study of Learning and Attention were used in this investigation (Supplementary Table S1). The 50 RD cases were identified by scores below the 25th percentile on either the Word Identification or Word Attack portions of the Woodcock Johnson III Achievement Battery or reading fluency on the Gray Oral Reading Test (GORT) (Wiederholt and Bryant, 1992; Woodcock, 1987; Woodcock and Johnson, 1989). The 25 non-impaired controls were defined as subjects with scores above the 40th percentile on the same tasks. Among subjects, there were 51 males and 31 females, with a mean age of 8.8 years (range 7–12 years). Exclusion criteria for subjects included IQ$<$85, left-handedness, hearing loss, severe articulation problems, severe emotional disturbance, autism, mental retardation, brain injury, neurologic disorders, and speaking English as a second language. Reading measures were performed on 75 subjects, imaging-reading data were collected on 82 subjects (51 males/31 females), and DNA was collected by buccal swab from subjects and all available family members (n = 326). All subjects gave informed consent approved by the Human Investigation Committee of the Yale University School of Medicine, and all studies were performed in accordance with the Declaration of Helsinki.

Behavioral Measures of IQ and Reading Performance

Verbal, performance and full scale IQ were determined by the Wechsler Abbreviated Scale of Intelligence (WASI) (Psychological Corporation, 1999). A range of quantitative neurobehavioral tasks measured reading performance of subjects. The Woodcock Johnson III Achievement Battery determined basic reading, letter-word identification, word attack, passage comprehension, and spelling (Woodcock, 1987; Woodcock and Johnson, 1989). The GORT assessed reading rate, accuracy, fluency, and comprehension (Wiederholt and Bryant, 1992). The Test of Word Reading Efficiency (TOWRE) measured sight word efficiency, phonemic decoding efficiency and total word efficiency (Torgesen et al., 1999). The Comprehensive Test of Phonological Processing (CTOPP) measured phonological awareness ability, blending words, memory for digits, rapid digit naming, blending of non-words, and segmenting non-words (Wagner et al., 1999). Behavioral measures are compiled in Supplementary Table S1, and correlations between tests are shown in Supplementary Table S2.

Imaging-Reading Tasks

Subjects completed four reading tasks while being imaged: Word Rhyming (WR)/Non-Word Rhyming (NWR) and Print Categorization (PC)/Auditory Categorization (AC) (Landi et al., 2010; Pugh et al., 2008; Shaywitz et al., 1998). In the WR/NWR tasks, subjects were asked to match a printed target consisting of a word (WR) or pseudoword (NWR) to a pictorially presented cue. A small set of cue pictures was used for both tasks consisting of pictures of either living or nonliving things. Subjects observed the cue picture, while a target stimulus was printed just below. Subjects were instructed to press one button if the cue and target stimulus rhymed, another if they did not. The WR/NWR tasks measured brain activation patterns involved in phonological processing. Phonological processing refers to the detection and separation of specific phonemes or speech sounds within words. Phonological processing is essential to reading and impaired in individuals with RD (Pugh et al., 2001; Shaywitz et al., 1999).

In the PC/AC tasks, subjects were asked to match either a printed (PC) or spoken (AC) target to a pictorially presented cue of living and non-living things. Subjects were instructed to press one button if the two stimuli were in the same category and another if they were not. For instance, “pig” and a picture of a fox are in the same category because both are living. The PC/AC tasks measure the brain activation patterns involved in semantic processing. Semantic processing refers to how one connects written and/or spoken words to actual language meaning. Individuals with RD can have deficits in semantic processing, yielding inaccurate comprehension of written language (Pugh et al., 2001).