Molecular Genetic Basis of Opposite Gene by Environment Interactions in Reading Disability and Attention Deficit/Hyperactivity Disorder

Jennifer Rosenberg
University of Denver

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Molecular Genetic Basis of Opposite Gene by Environment Interactions in Reading Disability and Attention Deficit/Hyperactivity Disorder

A Dissertation
Presented to
The Faculty of Social Sciences
University of Denver

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
Jennifer Rosenberg, M.A.

August 2011
Advisor: Bruce Pennington, Ph.D.
Abstract

The goal of this study is to better understand the genetic basis of Reading Disability (RD) and Attention Deficit Hyperactivity Disorder (ADHD) by examining molecular G x E interactions with parental education for each disorder. Research indicates that despite sharing genetic risk factors, RD and ADHD are influenced by different types of G x E interactions with parental education – a diathesis stress interaction in the case of ADHD and a bioecological interaction in RD. In order to resolve this apparent paradox, we conducted a preliminary study using behavioral genetic methods to test for G x E interactions in RD and the inattentive subtype of ADHD (ADHD-I) in the same sample of monozygotic and dizygotic Colorado Learning Disabilities Research Center same-sex twin pairs (DeFries et al., 1997), and our findings were consistent with the literature. We posited a genetic hypothesis for this opposite pattern of interactions, which suggests that only genes specific to each disorder enter into these opposite interactions, not the shared genes underlying their comorbidity.

This study sought to further investigate this paradox using molecular genetics methods. We examined multiple candidate genes identified for RD or related language phenotypes and those identified for ADHD for G x E interactions with parental education.
education. The specific aims of this study were as follows: 1) partition known risk alleles for RD and/or related language phenotypes and ADHD-I into those which are pleiotropic and non-pleiotropic by testing each risk allele for association with both RD and ADHD-I, 2) explore the main effects of parental education on both RD and ADHD-I, 3) address G-E correlations, and 4) conduct exploratory G x E interaction analyses in order to test the genetic hypothesis.

Analyses suggested a number of pleiotropic genes that influence both RD and ADHD; however, results did not remain after correcting for multiple comparisons. Although exploratory G x E interaction findings were not significant after multiple comparison correction, results suggested a G x E interaction in the bioecological direction with KIAA0319, parental education, and ADHD-I. Given the limited power in the current study, replication of these findings with larger samples is necessary.
Acknowledgments

This work was supported in part by two grants from the NICHD (HD-27802 and HD-49027). I would like to acknowledge the staff members of the many Colorado school districts, and the twins and their families who participated in this research.

I would like to express my deepest gratitude to Dr. Bruce Pennington for his guidance, patience, and support throughout my graduate training. I would also like to thank my committee members, Drs. Erik Willcutt, Ben Hankin, and Cynthia McRae, as well as Drs. Shelley Smith, Matt McQueen, and Lauren McGrath for their support and intellectual insight. In addition, I would like to express my gratitude to the many individuals who provided instrumental support throughout this project, particularly Laura Santerre-Lemmon, Dr. Holly Barnard, and my University of Denver cohort.

Finally, I would like to thank my family for their unwavering love, support, and encouragement. I would particularly like to acknowledge my grandmother who was with me during every step of this process.
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INTRODUCTION

Reading disability (RD) is a common neurodevelopmental disorder with defining symptoms including deficits in accurate and fluent word recognition (International Dyslexia Association [IDA], 2002). Numerous epidemiological studies have substantiated the familiality of RD (e.g. Gilger, Borecki, & DeFries, 1991), and modern twin studies have confirmed the substantial genetic etiology of this disorder (e.g., DeFries & Gillis, 1993). Attention Deficit Hyperactivity Disorder (ADHD) is also a common familial and heritable neurodevelopmental disorder (e.g., Biederman, Faraone, Keenan, Knee, & Tsuang, 1990; Faraone, Biederman, Keenan, & Tsuang, 1991; Gjone, Stevenson, & Sundet, 1996; Sherman, Iacono, & McGue, 1997; APA, 1994, 2000) with defining symptoms categorized into 3 subtypes: primarily inattentive, primarily hyperactive-impulsive (H-I), and combined (both inattentive and H-I) symptomatology.

OVERVIEW OF RD AND ADHD: GENES AND ENVIRONMENTS

GENETIC CONTRIBUTIONS TO RD

Due to advances in behavioral and molecular genetics, the understanding of the etiology of RD has advanced considerably in the past few decades (e.g. DeFries & Alarcón, 1996; Light & DeFries, 1995; Stevenson, 1993). Numerous epidemiological studies have substantiated the familiality of RD (e.g. Gilger et al., 1991). Approximately 30%-50% of children whose parents have RD will also develop the disorder, which is a
relative risk of about four to eight times that of controls (Gilger et al., 1991; Pennington & Lefly, 2001, Pennington, 2002). Modern twin studies have confirmed that most of the familiality of RD is genetic, with the remaining variability due to shared and non-shared environmental factors (DeFries & Gillis, 1993). One study using a large twin sample found a heritability estimate of .58, indicating that over half of the reading deficit variance is due to genetics (Wadsworth, Olson, Pennington, & DeFries, 2000).

Many studies have replicated the findings that reading ability lies on a continuum of a normal distribution (Rodgers, 1983; Shaywitz, Escobar, Shaywitz, Fletcher, and Makuch, 1992). This indicates that reading and its cognitive correlates are quantitative traits. Thus, the cognitive correlates that underlie high reading ability also underlie low reading ability (Boada, Willcutt, Tunick, Chhabildas, Olson, DeFries, and Pennington, 2002). The etiology of quantitative traits is often multifactorial, consisting of multiple genetic and environmental effects, in addition to the combination of the two. Genetic factors that affect such multifactorial traits are called quantitative trait loci (QTLs).

Additionally, through the use of genetic linkage and association studies, several linkage peaks and candidate genes have been identified that are associated with RD (e.g., Fisher and DeFries, 2002). See Table 1 for previously identified RD loci. Of note, four candidate genes (DYX1C1 in the 15q21 region, KIAA0319 in the 6p22 region, DCDC2 in the 6p22 region, and ROBO1 in the 3p12 region) for developmental dyslexia that affect neuronal migration and axonal guidance in brain development have been identified; however, replication of these results has been variable (for a review see McGrath, Smith, & Pennington, 2006; Fisher & Francks, 2006). As noted in Table 1, the
current study focuses on the following candidate genes: KIAA0319, DYX1C1, DCDC2, and ROBO1. In addition, given the comorbidity of speech/language and reading disorders, the following genes associated with speech and language development will also be included in the current study: CMIP, CNTNAP2, ATP2C2, and FOXP2. Of

<table>
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<tr>
<th>Table 1. Previously Identified RD Risk Genes</th>
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<tr>
<td>1p36-p34+</td>
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<td>(Rabin, Wen, Hepburn, Lubs, Feldman, and Duara, 1993; Grigorenko, Wood, Meyer, Pauls, Hart, and Pauls, 2001; Tzenova, Kaplan, Petryshen, and Field, 2004; Zhou et al., 2008)</td>
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<tr>
<td>2p10-15</td>
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<td>(Fagerheim, Raeymaekers, Tonnessen, Pedersen, Tranebjaerg, and Lubs, 1999; Fisher et al., 2002; Francks et al., 2002; Petryshen, Kaplan, Hughes, Tzenova, and Field, 2002; Kaminen, Hannula-Jouppi, Kestila, Lahermo, Muller, and Kaaranen, 2003; Peyrard-Janvid et al., 2004)</td>
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<tr>
<td>3p12-q13+</td>
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<td>(Fisher et al., 2002; Nopola-Hemmi et al., 2001)</td>
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<td>4q12-13+</td>
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<td>(Fisher et al., 2002)</td>
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<tr>
<td>6p22.2*+</td>
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<tr>
<td>(KIAA0319*+, DCDC2*+)</td>
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<td>(Fisher et al., 2002; Smith, Kimberling, and Pennington, 1991; Cardon, Smith, Fulker, Kimberling, Pennington, and DeFries, 1994; Cardon, Smith, Fulker, Kimberling, Pennington, and DeFries, 1995; Grigorenko et al., 1997; Fisher et al., 1999; Gáyan et al., 1999; Grigorenko, Wood, Meyer, and Pauls, 2000; Kaplan et al., 2002; Turic et al. 2003; Marlow et al., 2003; Grigorenko et al., 2003; Francks et al., 2004; Cope et al., 2005; Deffenbacher et al., 2004; Cuoto et al., 2009; Ludwig et al., 2008; Schumacher et al., 2006; Cuoto et al., 2009; Meng et al., 2005; Wilcke et al., 2009; Ludwig et al., 2008; Lind, Luciano, Wright, Montgomery, Martin, and Bates, 2010; Galaburda, LoTurco, Ramus, Fitch, and Rosen, 2006)</td>
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<tr>
<td>7q31-7q32</td>
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<tr>
<td>(FOXP2*)</td>
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<td>(Kaminen et al., 2003; Lai et al., 2003; O’Brief et al., 2003; Fenk et al., 2006)</td>
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<tr>
<td>8p23</td>
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<tr>
<td>(Fisher et al., 2002)</td>
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<tr>
<td>15q15-15q21+</td>
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<tr>
<td>(DYX1C1*)</td>
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<tr>
<td>(Taipale et al., 2003; Chapman, et al., 2004; Marino et al., 2004; Smith, et al., 1991; Grigorenko et al., 1997; Nothen et al., 1999; Smith, Kimberling, Pennington, and Lubs, 1983; Fulker et al., 1991; Nopola-Hemmi, Taipale, Haltia, Lehesjoki, Voutilainen, and Kere, 2000; Morris et al., 2000; Schulte-Korne et al., 1998)</td>
</tr>
<tr>
<td>8p11.2</td>
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<tr>
<td>(Fisher et al., 2002; Marlow et al., 2003; Fisher et al., 2002; de Kovel, Hol, Heister, Willemen, Sandkuijl, Franke, et al., 2004)</td>
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<tr>
<td>16p13+</td>
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<tr>
<td>(Loo et al., 2004)</td>
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<tr>
<td>17p11-q22+</td>
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<tr>
<td>(Loo et al., 2004)</td>
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<tr>
<td>ROBO1*</td>
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<td>(Hannula-Jouppi et al., 2005)</td>
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* indicates candidate genes that were included in the current study
+ indicates genes that have been shown to be pleiotropic with ADHD in previous studies
note, in addition to language development, Lesch and colleagues (2008) found an association between the ATP2C2 gene and ADHD.

**GENETIC CONTRIBUTIONS TO ADHD**

Unlike the majority of RD candidate genes that appear to be related to aspects of the development of brain structure, such as neuronal migration (e.g., Taipale, Kaminen,

<table>
<thead>
<tr>
<th>Table 2. Previously Identified ADHD Risk Genes</th>
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<tr>
<td>9-repeat (440 bp) and 10-repeat (480 bp) alleles of a 40-base pair variable number tandem repeat (VNTR) polymorphism in the 3’-untranslated region (UTR) of DAT1*</td>
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<tr>
<td>A1 and A2 alleles of a Taq1 polymorphism in intron 5 of DBH and a dinucleotide repeat on the 5’ end</td>
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<tr>
<td>A1 and A2 alleles of a Taq1 polymorphism of DRD2*</td>
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<tr>
<td>7-repeat allele of VNTR polymorphism in exon 3 of DRD4*</td>
</tr>
<tr>
<td>148 bp dinucleotide repeat 18.5 kb 5’ of DRD5*</td>
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<tr>
<td>ADRA2A (promoter region and region near ADRA2C)*+</td>
</tr>
<tr>
<td>44 bp insertion/deletion in promoter region of 5-HTT*</td>
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<td>861G allele of HTR1B</td>
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<tr>
<td>SNPs at positions 1065 and 1069 of SNAP-25</td>
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</table>
Despite a number of studies indicating association of RD and ADHD with various alleles, the molecular genetics literature for both disorders is mixed, particularly due to non-replication of results. For example, the current ADHD literature suggests an association between DAT1 and ADHD. Although a number of studies have found this association (Cook et al., 1995; Gill et al., 1997; Waldman et al., 1998; Daly et al., 1999; Barr et al., 2001; Curran et al., 2001; Chen et al., 2003), many studies have failed to replicate this relationship (Asherson et al., 1998; Palmer, et al. 1999; Holmes, Payton, Barrett, Hever, Fitzpatrick, and Trumper, 2000; Swanson et al., 2000; Todd, Jong, Lobos, Reich, Heath, and Neuman, 2001; Muglia, Jain, Inkster, and Kennedy, 2002). In 2007, Yang and colleagues performed a meta-analysis to further investigate the association between the 10 repeat allele of a VNTR polymorphism in the 3’ untranslated region of the DAT1 gene and ADHD. Findings suggested a small but significant association.
between the DAT1 polymorphism and ADHD when considering only the studies that used transmission disequilibrium tests (TDT) but did not find a significant association when considering the studies that either used case-control methodology or that used haplotype-based relative risk methodology. Similar non-replication findings exist within the RD literature, as well. Mixed findings may be due to a lack of statistical power, sample biases, different study methodologies, and also due to the heterogeneity of both disorders (Yang et al., 2007). Similarly, although the DRD4-7R has previously been associated with ADHD in the literature, a recent study by Bidwell et al., (2011) found an association between ADHD and the 4R allele in a similar subsample of CLDRC participants to that used in this study.

These mixed results underscore the need for further research investigating not only the association of various candidate genes with both disorders but also the investigation of G x E interactions involving the identified candidate genes, as some genes may only be associated with each disorder under certain environmental conditions. The current study will not only test for environmental and genetic main effects influencing RD and ADHD but also test for G x E interactions affecting both disorders.

**Comorbidity of RD and ADHD**

RD and ADHD co-occur in approximately 15-40% of cases (e.g., Gilger, Pennington, and DeFries, 1992; Shaywitz, Fletcher, and Shaywitz, 1995; Willcutt and Pennington, 2000; Willcutt, Pennington, Olson, and DeFries, 2007). The best supported hypothesis for the comorbidity between RD and ADHD is that they partially share
genetic risk factors. These shared genetic risk factors therefore exhibit pleiotropy because they influence more than one phenotype (e.g., Gayán et al., 2005; Willcutt et al., 2007). Willcutt and colleagues (2005) demonstrated that the genetic correlation ($r_g$) between RD and ADHD ranged from .37 to .70, indicating that the same genes at least moderately influence both disorders.

Using a molecular genetics approach, Gayán and colleagues (2005) presented further evidence that RD and ADHD are comorbid, in part due to pleiotropic genes. This study was the first to test for genome-wide linkage analysis, using a sib-pair sample (182 sib pairs) selected for RD but also meeting criteria for DSM-III ADHD (APA, 1987). Bivariate linkage was assessed using the D-F regression model (DeFries and Fulker 1985, 1988; Fulker et al., 1991). Results found evidence for pleiotropy for loci on chromosomes 14q32, 13q32, and 20q11 (Gayán et al., 2005). Prior to this study, only one study used bivariate linkage to test for pleiotropic genes underlying RD and ADHD (Willcutt et al., 2002). The previous study found linkage for many ADHD and RD phenotypes, suggesting pleiotropic effects; however, the linkage analysis was confined to a particular region implicated for RD on chromosome 6p21. Willcutt et al. (2002) demonstrated ADHD linkage to a previously identified region for RD (between markers D6S276 and D6S105) and also found bivariate linkage to marker D6S105.

Further, several studies have identified a number of overlapping linkage and association regions for RD and ADHD using univariate methods, suggesting common genes underlying both disorders (e.g., Bakker et al., 2003; Ogdie et al., 2004; Loo et al., 2004; Cuoto et al., 2009). For example, Loo and colleagues (2004) tested for RD
susceptibility loci in a sample originally ascertained for ADHD (aged 5-18 years old). Results indicated potential pleiotropic loci influencing RD and ADHD on chromosomes 2p and 15q, loci that have been previously implicated for RD (e.g., Fagerheim et al., 1999; Schulte-Korne et al., 1998; Grigorenko et al., 1997). Loo et al. (2004) also found evidence for RD linkage to loci in the 16p, 17q, and 10q regions; however, mixed results have been found across studies (e.g., Bakker, 2003), highlighting the importance of further investigating and replication. In 2003, Bakker et al. also found a significant linkage peak in region 15q, a region previously implicated for RD (Grigorenko et al., 1997; Nothen et al., 1999) in a sample originally ascertained for ADHD. Further, Barr et al., (2000) found significant association with RD and marker D15S146, one of the main contributing markers in the Bakker et al. (2003) findings, indicating that region 15q potentially underlies RD and ADHD.

A recent study by Cuoto and colleagues (2009) investigated whether markers in two regions on 6p22 (VMP/DCD2 and KIAA0319/TRAPP), previously associated with RD, were also associated with ADHD. More specifically, this study tested for association with the following markers and haplotypes: rs793862-rs807701 in DCD2 (Meng, Smith, Hager, Held, Liu, Olson, et al. 2005; Schumacher et al., 2006), rs4504469-rs2038137-rs2143340 in KIAA0319, TTRAP (Francks, Paracchini, Smith, Richardson, Scerr, Cardon, et al., 2004; Harold, Paracchini, Scerri, Dennis, Cope, Hill, et al., 2006; Luciano, Lind, Duffy, Castles, Wright, Montgomery, et al., 2007), and rs4504469-rs6935076 in KIAA0139 (Cope et al., 2005; Harold et al., 2006; Luciano et al., 2007) with ADHD. Results indicated significant association of ADHD affection and ADHD
symptom dimensions in the DCD2/VMP region, particularly at markers rs793862 and rs807701. Association was also found with markers in the VMP region and ADHD but results were less consistent with markers in this region compared to the DCD2 region. Further, association of ADHD to the KIAA0319/TTRAP region was weaker and less consistent. Although further investigation and replication of these results is necessary to determine whether the aforementioned markers are potentially pleiotropic candidate genes underlying ADHD and RD, this study provides further evidence that the 6p22 locus influences both disorders.

Further, in 2005, Stevenson and colleagues tested whether noradrenergic (NA) mechanisms were responsible for the [genetic] comorbidity of RD and ADHD (Kornetsky, 1970). The NA hypothesis posits that the ADRA2A marker in the 10q24-26 region plays a role in working memory that underlies the cognitive deficits observed in RD and ADHD. Studies have shown that children with comorbid RD and ADHD demonstrate elevated levels of an NA metabolite, 3-mehoxy-4-hydroxyphenylglycol (MHPG; Halperin, Newcorn, Koda, Pick, McKay, and Knott, 1997; Bonafina, Newcorn, McKay, Koda, and Halperin, 2000). The NA hypothesis, however, has garnered mixed support in the molecular genetics literature for RD and ADHD. For example, Barr et al. (2002, 2001) found null results when testing for the association of NET1, a norepinephrine transporter gene, and ADHD. Null results were also observed when testing two adrenergic receptor loci (ADRA1C in region 8p11.2 and ADRA2A in region 4p16) and ADHD. Similarly, Xu et al. (2001) found null results when testing for linkage and association between ADRA2A and ADHD. On the other hand, a number of studies
have replicated positive associations between variations of ADRA2A and ADHD (Roman, Schmitz, Polanczyk, Eizirik, Rohde, & Hutz, 2003; Comings, Gade-Andayolu, Gonzalez, Wu, Muhleman, Blake, et al. 2000; Park, Nigg, Waldman, Nummy, Huang-Pollock, Rappley, et al., 2005). Additionally, studies testing association for ADRA2A variants and RD without comorbid ADHD have not produced significant results (see Fisher and DeFries, 2002 for a review). In an attempt to reconcile the mixed findings in the literature regarding the influence of the NA system on comorbid ADHD and RD, Stevenson et al. (2005) tested for linkage and association of a variant of ADRA2A and comorbid ADHD + RD. Results showed significant association between the ADRA2A variant and comorbid ADHD + RD. Given the inconsistent findings when testing for association between ADRA2A and ADHD (without comorbid RD) and the lack of significant association findings between ADRA2A and RD (without comorbid ADHD), Stevenson et al. (2005) suggest that individuals with comorbid ADHD + RD may represent a distinct clinical group that exhibits association with adrenergic gene variants.

Overall, although there is evidence for pleiotropic genes underlying RD and ADHD, given the mixed literature, further research and replication using samples ascertained for both RD and ADHD is necessary to substantiate findings. Further, in addition to shared genes, there is some evidence for genes specific to each disorder (e.g. Gayán et al., 2005, Gilger et al., 1992; Light, Pennington, Gilger, & DeFries, 1995; Light & DeFries, 1995; Willcutt & Pennington, 2000; Willcutt et al., 2007). For example, Gayán and colleagues (2005) did not find evidence of bivariate linkage to DYX7 or DYX8, indicating that these loci may only affect RD and not affect ADHD.
Much research has focused on four broad environmental factors that affect reading ability: home literacy environment, socioeconomic status (SES), family educational values, and home language stimulation (for a review see Phillips & Lonigan, 2005). Clearly, these variables have substantial overlap, but they also individually contribute to reading skill. Some controversy exists regarding to what extent home literacy environment, specifically shared book reading between parents and children, contributes to emergent literacy and language development (Scarborough & Dobrich, 1994a; Scarborough & Dobrich, 1994b; Lonigan, 1994; Dunning, Mason, & Stewart, 1994).

These varied results are in part due to methodological differences. Specifically, factors such as sample age, data analytic methods used, and measurement methods for the frequency and quality of shared book reading varied across studies. Additionally, only a small number of studies provided specific measurements of shared book reading quality and parental reading style, so other studies may have missed correlations between different aspects of shared reading and language development. For example, highlighting the importance of precise measurement regarding the quality of shared book reading, Sénéchal and colleagues (Sénéchal, LeFevre, Thomas, & Daley, 1998) found that informal parent-child storybook exposure predicted future receptive language skills, whereas a more formal, teaching approach to shared book reading predicted emergent literacy skills in children in kindergarten and first grade. Further, shared book reading
has been shown to have differential effects dependent upon children’s ages (Bus, van Ijzendoorn, & Pellegrini, 1995; Scarborough & Dobrich 1994b). The beneficial outcomes of joint book reading may be more pronounced in children of younger ages or rather, the effects of shared book reading may correlate to specific language skills at different age ranges. Furthermore, confounds, such as parent interest in reading that in turn may affect children’s joint book reading experiences, were not included in these studies.

Despite these inconsistent findings, studies agree that future research implementing more precise methodology is necessary to further elucidate the effects of shared reading. Additionally, many studies recognize the importance of investigating joint reading in a broader context of literacy development, and recommend using a model that includes multiple variables, such as other household activities that may also contribute to reading and language development (Scarborough & Dobrich, 1994b; Bus et al., 1995).

In 2008, Friend and colleagues found an environmental main effect of parental education on RD in a subsample of CLDRC participants, indicating a relationship between parental education and child reading apart from the well established familiality of RD. We replicated these findings in a preliminary study in a similar subset of CLDRC twins. Similar to Friend et al.’s (2008) results, this environmental main effect remained after controlling for parental retrospective self-reports of RD symptomatology. Building on the results of Friend et al. (2008) and on our preliminary research, the current study will focus on the effects of parental education on RD and ADHD.
ENVIRONMENTAL CONTRIBUTIONS TO ADHD

Many bioenvironmental and psychosocial variables have been studied for ADHD, but this research has resulted in only a few well-replicated environmental risk factors. Numerous correlational studies have shown relationships between various psychosocial variables and ADHD. These factors include early exposure to television (Christakis, Zimmerman, DiGiuseppe, & McCarty, 2004), environmental adversity, such as family conflict and low SES (Biederman, Milberger, Faraone, Kiely, Guite, Mick, Ablon, et al., 1995a; Biederman et al., 1995b; Biederman, Faraone, & Monuteaux, 2002a), and exposure to adult ADHD, although this variable may be genetically moderated (Biederman et al., 2002b). Many of these studies controlled for some confounding factors, such as parental psychopathology, parental substance use/abuse, and gestational age; however, most studies did not control for G-E correlations (Thapar et al., 2003). Hence, a more genetically sensitive design, such as a twin study, would help determine if these psychosocial correlates are actually environmental risk factors for ADHD.

Various bioenvironmental factors have also been shown to influence the development of ADHD. These factors include lead poisoning, pediatric head injury (for review see Barkley, 1996), older maternal age of delivery (Claycomb, Ryan, Miller, & Schnakenberg-Ott, 2004), birth weight (Hultman, Torrang, Tuvblad, Cnattingius, Larsson, & Lichtenstein, 2007), season of birth (Seeger, Schloss, & Schmidt, 2004) and prenatal alcohol and nicotine exposure (Barkley, 1996; Mick, Biederman, Faraone, Sayer, and Kleinman, 2002; Kotimaa et al., 2003; Thapar et al., 2003). Since lead exposure and
pediatric head injury account for a very small percentage of overall ADHD cases, research has mainly focused on pre- and peri-natal environmental factors (for review see Barkley, 1996). The effects of low birth weight (Siegel, 1982; Milberger, Biederman, Faraone, Guite, and Tsuang, 1997; Bhutta, Cleves, Casey, Cradock, and Anand, 2002; Claycomb et al., 2004; Thapar, et al. 2005; Hultman et al., 2007) and prenatal maternal smoking (Siegel, 1982; Mick et al., 2002; Bhutta et al., 2002; Claycomb et al., 2004; Seeger et al., 2004; Brookes et al. 2006; Nigg, 2006; Kotimaa et al., 2003; Thapar et al., 2003; Langley, Rice, and van den Bree, 2005; Wakschlag, Leventhal, Pine, Pickett, and Carter, 2006) on ADHD have been the most consistently replicated across studies.

We conducted preliminary research, using behavioral genetics methods, which showed that parental education influenced twin ADHD (I) symptomatology, even after controlling for parent retrospective reports of ADHD (maternal and paternal self-reports of inattention). Interestingly, results indicated that parent ADHD history partially suppresses the relation between parent education and child ADHD. This relationship is in contrast to what was found for parent reading history, which partially mediates the relation between parent education and child reading.

**G x E Interactions in RD and ADHD**

Interestingly, despite the genetic overlap underlying the comorbidity of RD and ADHD, research suggests that the two disorders enter into opposite types of G x E interactions. Although there has not been a substantial amount of research regarding G x E interactions specific to RD, there is some evidence that such interactions follow a
bioecological model of interaction. This model posits that enriched environments will allow genetic heritability to become most apparent. Consequently, genetic differences are not as evident in risk environments (Turkheimer & Gottesman, 1991; Bronfenbrenner & Ceci, 1994). Bioecological G x E interactions have been identified with parental education and word recognition (Kremen, Jacobson, Xian, Eisen, Waterman, Toomey, et al., 2005) and also with parental education and reading in an equivalent CLDRC twin subsample to that used in the current study (Friend et al., 2008). Similar findings have also been documented regarding pre-literacy and language phenotypes and speech and sound disorder (SSD; McGrath Pennington, Willcutt, Boada, Shriberg, and Smith, 2007) and with IQ and SES (Turkheimer, Haley, Waldon, D’Onofrio, and Gottesman, 2003; Harden, Turkheimer, and Loehlin 2007).

On the other hand, research investigating G x E interactions in ADHD suggests a diathesis-stress model (Rende & Plomin, 1992). This model predicts that a diathesis (genetic vulnerability) in addition to an environmental stressor will increase the heritability of a disorder (Barlow and Durand, 2002; O’Connor, Caspi, DeFries, and Plomin, 2003; Zuckerman, 1999). For example, Laucht and colleagues (2007) examined the interaction between the risk allele of the dopamine transporter (DAT1) and psychosocial adversity factors measured by the Rutter Family Adversity Index, which assesses 11 adverse family factors, including low parental education, marital discord, unwanted pregnancy, and poor social support of parents (Rutter and Quinton, 1977). Despite the lack of a genetic main effect, a G x E interaction was demonstrated, such that individuals who were homozygous for the DAT1 risk allele (10R allele of the 40-bp
VNTR) and who were also exposed to greater levels of psychosocial adversity had higher rates of inattention and hyperactivity/impulsivity (p=.001-.015). Although a number of molecular genetics studies have demonstrated diathesis stress G x E interactions with ADHD when involving various ADHD risk genes and both psychosocial and bioenvironmental environments, inconsistencies remain in the literature (e.g. Retz et al., 2007; Kahn, Khoury, Nichols, and Lanphear, 2003; Neuman, Lobos, Reich, Henderson, Sun, & Todd, 2007; Seeger et al., 2004; Lasky-Su et al., 2007). For example, much of this research identifies G x E interactions affecting H-I and oppositional symptomatology, but there is little evidence of G x E interactions affecting inattention symptoms. Paradoxically, twin studies have shown that extreme symptoms of inattention are highly heritable regardless of H-I symptomatology, but the reverse does not hold true (Willcutt et al., 2000; Willcutt, 2007).

Additionally, since it is possible that parental education is in fact a distal variable for a more proximal environmental factor, such as parental literacy practices in the case of RD and psychosocial adversity in the case of ADHD, the current study will first test for G x E interactions involving RD and ADHD candidate genes and parental education. We will then test the proximal environment hypothesis by residualizing a parental literacy practice composite from the parental education variable. G x E interactions for both disorders involving the residualized parental education environment variable will then be analyzed to assess whether the opposite types of G x E interaction are due to the environmental effects described above. Since we expect parent literacy environment to influence RD and not ADHD, we expect the bioecological G x E interaction with RD to
decrease once parent literacy practices are residualized from the parental education environment. On the other hand, we do not expect the magnitude or the direction of the G x E interaction (diathesis-stress) with ADHD to change whether the parental education variable or the residualized parental education variable is included in the G x E interaction models. Unfortunately, we do not have measures of psychosocial adversity in this sample, so we can not apply this procedure in the other direction.

Further, many of RD and ADHD G x E studies, to date, have failed to address confounds related to gene-environment (G-E) correlations. The term gene-environment correlation refers to the fact that environments may be partially correlated with genetic factors (Kendler and Baker, 2007; Plomin, 1994; Plomin and Bergeman, 1991; Scarr and McCartney, 1983). If independent influences of the environment cannot be teased apart from those stemming from genetic influences, an apparent G x E interaction may actually be a G x G interaction.

PRELIMINARY STUDIES

Many of the analyses described in this study are intended to extend previously investigated G x E interactions findings between RD and parental education and ADHD and parental education. Although current literature indicates opposite types of G x E interactions with RD (bioecological G x E interaction) and ADHD (diathesis-stress G x E interaction), this had not previously been investigated in the same sample. Consistent with current literature, preliminary results indicated a bioecological G x E interaction involving RD and parental education, such that RD was more heritable in a higher
parental education environment. Conversely, findings showed a diathesis-stress $G \times E$ interaction with ADHD and parental education, such that ADHD was more heritable in a lower parental education environment. Further analyses sought to resolve this paradox by testing whether the pleiotropic or non-pleiotropic genes underlying RD and ADHD were involved in these opposite interactions. Preliminary results are reported below.

Participants were recruited through the Colorado Learning Disabilities Research Center (CLDRC; DeFries et al., 1997), with sample sizes varying from 85-408, depending on analysis. Children completed a battery of psychological tests, including a number of reading measures. Parents completed questionnaires regarding attention and school performance (see Methods).

Reading ability was assessed using a word recognition composite that was created using two relevant measures: the PIAT Word Recognition subtest (Dunn & Markwardt, 1970), an untimed single word reading test, and the Timed Word Recognition (TWR) subtest (Olson, Wise, Conners, Rack, & Fulker, 1989; Olson Forsberg, Wise, & Rack, 1994), a single-word reading test which implements a time limit. Probands who met criteria for RD were defined as those whose word recognition composite fell below 1.5 standard deviations from the control mean. ADHD probands were defined by affection status (e.g., a “diagnosis” of ADHD), according to the DSM-IV. Twins who met criteria for ADHD – Combined subtype or ADHD – Inattentive subtype were considered ADHD probands. An inattentive symptom composite was then created by calculating the mean severity of inattentive symptoms, using data from all available raters for each child. Parental education data was provided by self-reports.
The DeFries-Fulker (D-F; 1988) method for analyzing twin pairs on extreme traits was used to replicate previous findings of univariate and bivariate heritability for RD and ADHD (e.g., DeFries & Alarcón, 1996; Light, et al., 1995). Univariate and bivariate heritability estimates were replicated in the current sample, indicating significant

<table>
<thead>
<tr>
<th>Table 3. (Preliminary Studies) Main Effects of E: Parental Education on Child Reading</th>
<th>N</th>
<th>Pearson’s r</th>
<th>(2-tailed) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bivariate correlation</td>
<td>408</td>
<td>.308</td>
<td>.001*</td>
</tr>
<tr>
<td>Partial correlation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controlling for mother</td>
<td>346</td>
<td>.254</td>
<td>.001*</td>
</tr>
<tr>
<td>Report of RD symptomatology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial correlation</td>
<td>324</td>
<td>.250</td>
<td>.001*</td>
</tr>
<tr>
<td>Controlling for father</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Report of RD symptomatology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial correlation</td>
<td>147</td>
<td>.169</td>
<td>.001*</td>
</tr>
<tr>
<td>Controlling for mean of both</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Parents’ retrospective</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Report of RD symptomatology</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* denotes significant main effect detected

<table>
<thead>
<tr>
<th>Table 4. (Preliminary Studies) Main Effects of E: Parental Education on Child ADHD-I Symptomatology</th>
<th>N</th>
<th>Pearson’s r</th>
<th>(2-tailed) p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>408</td>
<td>.149</td>
<td>.003*</td>
</tr>
<tr>
<td>Partial correlation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controlling for mother retrospective</td>
<td>205</td>
<td>.202</td>
<td>.003*</td>
</tr>
<tr>
<td>Report of ADHD-I symptomatology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controlling for father retrospective</td>
<td>157</td>
<td>.186</td>
<td>.019*</td>
</tr>
<tr>
<td>Report of ADHD-I symptomatology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial correlation</td>
<td>216</td>
<td>.181</td>
<td>.007*</td>
</tr>
<tr>
<td>Controlling for mean of both parents’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrospective report of ADHD-I symptomatology</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* denotes significant main effect detected

univariate heritability of RD (N=283, B=.593, p<.001), significant univariate heritability of the inattentive symptom dimension of ADHD (N=171, B=.874, p<.001), and significant bivariate heritability when using RD probands to predict cotwin inattention (N=283, B=.447, p=.013) and also when using ADHD probands to predict cotwin reading (N=171, B=.352, p=.009).
Bivariate correlation analyses were used to evaluate the association between parental education and child reading in the current sample. Since significant results were found ($r=.308, p<.001$), partial correlations were applied to further test this main effect while controlling for maternal and paternal retrospective self-reports of RD symptomatology. Replicating Friend et al.’s (2008) findings, these correlations remained significant after controlling for parent retrospective RD symptomatology (RHQ), indicating a relation between parent education and child reading apart from the well-established familiality of reading skill (Table 3). Parent education was also related to the mean severity of child ADHD (I) symptomatology even after controlling for retrospective parent ADHD (Table 4).

The G x E analyses were conducted using the extended DeFries-Fulker (D-F) regression equation that incorporates a G x E interaction term:

$$C = B_1 P + B_2 r + B_3 e + B_4 Pe + B_5 re + K$$

C represents the cotwin’s phenotypic score (i.e. RD). P represents the proband’s phenotypic score, and r is the coefficient of relationship, which for the MZ twins will be 1, and for the DZ twins will be .5, due to the amount of shared genetics in the twin pairs. The term, “e” represents the pair-specific environment (parental education). The beta weight of interest in this equation is $B_5$, which tests for the significance of the G x E interaction. K represents the regression constant.

Replicating methods implemented by Friend et al. (2008), parent education data was residualized by proband reading scores using linear regression in order to partial out confounding genetic influences from the parental education environmental factor.
G x E analyses showed a significant interaction in the bioecological direction (Figure 1, Table 5), whereby RD was more heritable when parents completed more years of education. Analyses also found a G x E interaction with ADHD and parental education in the diathesis-stress direction (Figure 2, Table 6), indicating that ADHD was more heritable in the unfavorable parental education environment.

### Table 5. Univariate G x E Interaction RD and Parental Education

<table>
<thead>
<tr>
<th>N Probands (Double Entered)</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate G x E interaction with RD and Parental Ed. (Residualized with Child Reading)</td>
<td>283 (178)</td>
<td>.249</td>
<td>.103</td>
<td>2.41</td>
</tr>
<tr>
<td>(h^2_g) Reading in “High” Parental Ed. E</td>
<td>142 (81)</td>
<td>.736</td>
<td>.164</td>
<td>4.49</td>
</tr>
<tr>
<td>(h^2_g) Reading in “Low” Parental Ed. E</td>
<td>141 (97)</td>
<td>.459</td>
<td>.153</td>
<td>3.01</td>
</tr>
</tbody>
</table>

### Table 6. Univariate G x E Interaction ADHD-I and Parental Education

<table>
<thead>
<tr>
<th>N Probands (Double Entered)</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate G x E interaction with ADHD-I and Parental Ed. (Residualized with Child ADHD)</td>
<td>171 (74)</td>
<td>- .345</td>
<td>.171</td>
<td>-2.01</td>
</tr>
<tr>
<td>(h^2_g) ADHD-I in “High” Parental Ed. E</td>
<td>86 (30)</td>
<td>.557</td>
<td>.249</td>
<td>2.24</td>
</tr>
<tr>
<td>(h^2_g) ADHD-I in “Low” Parental Ed. E</td>
<td>85 (44)</td>
<td>1.16</td>
<td>.206</td>
<td>5.62</td>
</tr>
</tbody>
</table>

Since significant opposite G x E interactions were found with parental education for RD and ADHD using the same sample of twins, we hypothesized that there might be three overlapping genetic subtypes of probands: RD-only, ADHD-only, and RD+ADHD. Theoretically, we expected to find stronger G x E interaction results when testing parental education with RD-only (twins who met study criteria for RD but did not meet study criteria for ADHD) and ADHD-only probands (twins who met criteria for ADHD but did
Table 7. Univariate G x E Interaction RD and Parental Education in RD-Only Twins

<table>
<thead>
<tr>
<th>N Probands (Double Entered)</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate G x E Interaction RD and Parental Ed. E</td>
<td>197(121)</td>
<td>.266</td>
<td>.117</td>
<td>2.28</td>
</tr>
<tr>
<td>$h_g^2$ RD in “High” Parental Ed. E</td>
<td>98(52)</td>
<td>.782</td>
<td>.186</td>
<td>4.20</td>
</tr>
<tr>
<td>$h_g^2$ RD in “Low” Parental Ed. E</td>
<td>99(69)</td>
<td>.503</td>
<td>.190</td>
<td>2.64</td>
</tr>
</tbody>
</table>

not meet study criteria for RD), as these probands should have the genes that drive the opposite G x E interactions. Conversely, we expected to find weaker interaction results when testing G x E interactions with comorbid twins (twins who met study criteria for RD and ADHD), assuming the shared genes are not the genes driving the opposite interactions in RD and ADHD. As predicted, the bioecological interaction with parental education (residualized by twin reading to address potential G-E confounds and RD in “pure” RD probands was significant (Table 7).

Similarly, results found a diathesis-stress interaction with parental education (residualized by twin ADHD symptomatology) and ADHD in “pure” ADHD probands.
Interaction results for comorbid probands and parental education were non-significant for RD (Table 9) and ADHD (Table 10). On further examination of the G x E interactions described above, it became less clear whether the G x E interaction results with the “pure” proband groups were significantly different (stronger) than the G x E interactions observed with the comorbid RD and ADHD probands. If the interactions with the “pure” proband groups were stronger than those in the comorbid probands, we would expect that when the interactions were broken down by median splits into “high” and “low” parental education environments, the magnitude of the differences in the heritability estimates (e.g., the difference between the heritability estimate of RD in the high parental education environment and the heritability estimate of RD in the low

| Table 8. Univariate G x E Interaction ADHD and Parental Education in ADHD-Only Twins |
|----------------------------------|----------------|---|---|---|---|
| Univariate G x E Interaction ADHD and Parental Ed. E | N Probands (Double Entered) | B  | SE  | t   | p    |
| h\textsubscript{g} ADHD in “High” Parental Ed. E | 42 (12) | .512 | .366 | 1.40 | 0.085 |
| h\textsubscript{g} ADHD in “Low” Parental Ed. E | 43 (20) | 1.19 | .288 | 4.13 | <.001 |

| Table 9. Univariate G x E Interaction RD and Parental Education in Comorbid Twins |
|----------------------------------|----------------|---|---|---|---|
| Univariate G x E Interaction RD and Parental Education | N Probands (Double Entered) | B  | SE  | t   | p    |
| h\textsubscript{g} RD in “High” Parental Ed. E | 43 (27) | .697 | .327 | 2.13 | 0.020 |
| h\textsubscript{g} RD in “Low” Parental Ed. E | 43 (30) | .292 | .250 | 1.17 | 0.130 |
parental education environment) would be significantly greater in the pure proband groups compared to the comorbid twins. This pattern however, was not as clear as we anticipated. For example, it was unclear whether the difference between the heritability estimates in the high and low parental education environments in the “pure” RD probands (Table 7; B=.782 compared to B=.503) was significant while the difference between the heritability estimates in the high and low parental education in the comorbid RD probands (Table 9; B=.697 compared to B=.292) was not despite this pattern with the G x E interaction terms in both proband groups. In other words, based on the aforementioned analyses, it was unclear whether the G x E interaction with parental education in RD was qualified by ADHD.

Since the evidence for the pleiotropy hypothesis was less clear than one would expect given the pleiotropy hypothesis, we directly tested for the implied three-way interaction for RD and ADHD and parental education. The three-way interaction equation is as follows:

\[ C = B_1P + B_2r + B_3PE + B_4ADHD + B_5Proband*ADHD + B_6r*PE + B_7r*ADHD + B_8ADHD*PE + B_9r*PE*ADHD + K \]

Table 10. Univariate G x E Interaction ADHD and Parental Education in Comorbid Twins

<table>
<thead>
<tr>
<th></th>
<th>N Probands (Double Entered)</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate G x E Interaction ADHD-I and Parental Education</td>
<td>86 (42)</td>
<td>-.335</td>
<td>.277</td>
<td>-1.21</td>
<td>.115</td>
</tr>
<tr>
<td>( h_g^2 ) ADHD-I in “High” Parental Ed. E</td>
<td>43 (17)</td>
<td>.786</td>
<td>.352</td>
<td>2.23</td>
<td>.016</td>
</tr>
<tr>
<td>( h_g^2 ) ADHD-I in “Low” Parental Ed. E</td>
<td>43 (25)</td>
<td>.883</td>
<td>.294</td>
<td>3.00</td>
<td>.002</td>
</tr>
</tbody>
</table>

C represents the cotwin’s phenotypic score (i.e., RD in the example above). P represents the proband’s phenotypic score (i.e., RD in the example above), r is the coefficient of
relationship, which for the MZ twins will be 1, and for the DZ twins will be .5, due to the amount of shared genetics in the twin pairs. PE is the environmental term, parental education, and ADHD is the proband measure of the other phenotypic dimension (e.g., if the above equation example was intended to measure whether the G x E interaction with ADHD changes as a function of parent education and RD, the “ADHD” term would instead be proband RD). The beta weight of interest in this equation is $B_9$, which tests for the significance of the three-way interaction of whether the G x E interaction for RD (in the above example) changes as a function of ADHD and parental education. K represents the regression constant.

In other words, we tested whether the G x E interaction for RD and parental education changed as a function of proband comorbidity with ADHD. Similarly, we tested whether the G x E interaction for ADHD and parental education changed as a function of proband comorbidity with RD. Analyses produced null results for both three-way interaction tests, providing evidence that does not support the pleiotropy hypothesis. These mixed findings highlight the importance of further investigation into this paradox. The current study is intended to follow-up these preliminary results using molecular genetics methods to directly test the pleiotropy and differing proximal environment hypotheses with previously identified candidate genes for RD and ADHD. Further inquiry using molecular genetics methods will better be able to identify which genes are involved in the opposite types of G x E interactions with each disorder.
**Specific Aims**

The overall goal of this project was to advance understanding of the multifactorial etiology of RD and ADHD by examining the reasons for opposite G x E interactions in each disorder. This project fills a gap in the RD and ADHD genetic literature by focusing on G x E interactions using molecular genetics methods. The primary aims of this study were to 1.) partition known risk alleles for RD and ADHD into those that are pleiotropic and those that are non-pleiotropic using association methods, 2.) replicate and extend the current literature on the G x E interactions influencing RD and ADHD by testing the G x E interactions influencing RD and ADHD with parental education, and 3.) if opposite G x E interactions were found in the expected directions (e.g., bioecological for RD risk genes and parental education and diathesis-stress for ADHD risk genes and parental education), test two competing hypotheses to further investigate the paradox described above: a.) the opposite G x E interactions are due to non-pleiotropic risk alleles and not to pleiotropic ones and b.) the opposite G x E interactions are due to differing proximal environmental variables, such as parent literacy practices, that are nested under the broad environmental variable, parental education. Additionally, although previous studies have investigated G x E interactions influencing RD and ADHD, this study will be the first to implement molecular genetics methods to test for such G x E interactions in the same sample. The results of this study will inform theoretical models as well as
provide information that will inform future attempts at early identification and intervention for both disorders.
METHOD

PARTICIPANTS

The present study will be part of the ongoing Colorado Learning Disabilities Research Center Twin Project (CLDRC; DeFries et al., 1997), an ongoing study of the etiology of learning disabilities, ADHD, and other related disorders (e.g. DeFries et al., 1997). Participants were recruited from school districts in the Front Range area, and parents of all twins between the ages of 8 and 18 within the selected school districts were contacted via letter and invited to participate in the study. After initial parental consent was obtained, two parallel recruitment processes were conducted independently to identify twin pairs in which at least one of the twins met criteria for ADHD or at least one of the twins exhibited significant reading difficulties. Twin pairs in which neither twin met criteria for either ADHD or reading difficulties were recruited to participate for inclusion into a comparison group.

For the purposes of this study, a sample of monozygotic (MZ) and dizygotic (DZ) twin pairs were recruited from this aforementioned twin sample. To identify twin pairs in which at least one twin met criteria for significant reading or attentional difficulties, parental consent was requested to allow study staff to review each twin’s academic record. If either member of the twin pair had a positive history of academic difficulties (i.e. low achievement test scores, referral to a tutor, reports by classroom teachers or
school psychologists) or ADHD, both twins were invited to participate in the study. Twin pairs in which neither twin had a history of reading difficulties or ADHD were invited to take part in the larger study and were then included in the comparison sample.

The CLDRC staff conducted a telephone screening interview prior to any testing. Exclusion criteria and study parameters have been described previously (DeFries et al., 1997). Participants were excluded from this study if they had evidence of a significant neurological (e.g. seizures), emotional, or behavioral (e.g. autism) disorder, had evidence of a known genetic syndrome (e.g. sex chromosome aneuploidy), or had any uncorrected visual or auditory acuity deficits. Control twins were matched to affected twins on the basis of age, gender, and school district.

**Measures**

<table>
<thead>
<tr>
<th>Reading</th>
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</table>

A word recognition composite measure was created using the Peabody Individual Achievement Test- Word Recognition (Dunn & Markwardt, 1970) and the Timed Word Recognition (Olson, Wise, Conners, Rack, & Fulker, 1989; Olson, Forsberg, Wise, & Rack, 1994) tests. Olson and colleagues (2003) demonstrated, via structural equation modeling (SEM), that the PWR and TWR subtests created a coherent composite measuring the latent variable of word recognition in the CLDRC twin sample. Therefore, a composite was created by averaging the age standardized raw scores from the PWR and TWR tasks.
1.) **Peabody Individual Achievement Test – Word Recognition (PWR; Dunn & Markwardt, 1970):** The PIAT is an individually administered achievement test, intended for children and adolescents in kindergarten through grade 12. The Word Recognition subtest requires individuals to read across rows of increasingly difficult, unrelated words until they reach an error criterion of five errors in the last seven words presented. The final score is based on the number of correct items. There is no time constraint for this measure. The published test-retest reliability for this measure is .96 (Dunn & Markwardt, 1970).

2.) **Timed Word Recognition (TWR) Test** (Olson, et al., 1989; Olson et al., 1994):

The TWR assesses word recognition accuracy and processing speed. Lowercase words are presented in order of difficulty on a computer screen. Participants are initially placed at varying starting levels of difficulty based on a 14-item screening list. Children are instructed to “…read the words as quickly as you can without making mistakes,” and to “…sound it out or give it a good guess” for unknown words. Participants have to respond within two seconds after the appearance of the word on the screen, as indicated by a voice key in order for the item to be scored as correct. Test progression is terminated when the participant fails to read ten of the last 20 items correctly within the 2-second response limit. The TWR is composed of a total of 182 isolated words. A test-retest correlation of .93 was obtained with an independent sample of 123 third- through sixth-grade poor readers across four months (Olson et al., 1994).
1. **Attention Deficit Hyperactivity Disorder Rating Scale-IV** (ADHDRS-IV, DuPaul et al., 1998): The ADHDRS-IV is a questionnaire probing for DSM-IV criteria for ADHD, intended for children aged 5 through 18 years. The ADHDRS-IV is composed of 18, 4-point Likert scale items (1 = never or rarely, 2 = sometimes, 3 = often, 4 = very often). The first nine questions of the ADHDRS-IV probe for Inattention symptoms, and the second set of nine items probes for Hyperactivity-Impulsivity symptoms. The ADHDRS-IV was completed by participants’ parents, and teachers, providing as many as three raters for each individual. Children were diagnosed as ADHD if they demonstrated six or more symptoms of inattention, six or more symptoms of hyperactivity/impulsivity, or six or more in both domains, rated by either a parent or teacher. Participants were further classified as either ADHD-Combined Type (ADHD-C), ADHD-Inattentive Type (ADHD-I), or ADHD-Hyperactive/Impulsive (ADHD-HI) in accordance with DSM-IV diagnostic criteria. On the home version of the ADHDRS-IV, test-retest correlations of .85 for total score, .78 for inattention, and .86 for hyperactivity-impulsivity were obtained. On the school version of the scale, test-retest correlations of .90 for total score, .90 for inattention, and .88 for hyperactivity-impulsivity were found (DuPaul et al., 1998).

<table>
<thead>
<tr>
<th>Objective Home Environment</th>
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</table>

1. **Parental Level of Education**: Parental level of education is often used as a marker variable for SES (Smith, Brooks-Gunn, Klebanov, 1997). Maternal and paternal level of education was obtained by self-report. A variable for mean years of parental education.
was computed if both maternal and paternal education data is provided. In cases where only maternal or paternal education is provided, only that parent’s data is included in analyses.

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**Retrospective Reports of Parental Reading and Attention**

1.) **Parental Reading History Questionnaire** (RHQ; Lefly & Pennington, 2000): Both parents completed the RHQ, which includes Likert-scale items asking them to recall and assess their attitude toward and experience with reading and academics as children.

2.) **Retrospective ADHD Interview for Parents**: Parents provided information regarding their experiences of inattention and hyperactivity/impulsivity as a child before the age of 12.

**PROCEDURES**

*Initial Recruitment and CLDRC Data Collection*

Parents provided consent for their children to participate in the behavioral portion of this study, and children provided assent. Whenever possible, biological siblings of the twin pair that are within the 8-18 age range were also tested. As part of the larger CLDRC study, all twins and their siblings completed a psychoeducational battery at the University of Colorado and the University of Denver that included measures of general cognitive ability, reading, language, executive functioning, and other measures of neuropsychological functioning relevant to RD and ADHD. Additionally, both parents and children completed a series of interviews and self-report checklists covering a wide
array of psychosocial symptomatology. Teachers provided measures of child classroom performance and attention. The children were paid $100 and the parents were paid $20 for their participation in the study.

RD probands were defined as those whose word recognition composite scores (i.e., PWR and TWR) fell at least 1.5 standard deviations below the recruited control mean.

Research has shown that the ADHD-HI subtype has a different etiology than the ADHD-I subtype (Willcutt et al., 2006; Nikolas and Burt, 2010). Furthermore, preliminary analyses, based on a similar subsample of CLDRC participants, did not show significant bivariate heritability between ADHD-HI and RD but showed significant bivariate heritability findings between ADHD-I and RD. Therefore, based on exploratory and confirmatory factor analyses previously applied to the ADHDRS-IV scores from the CLDRC sample (McGrath et al., 2011), the final group of ADHD probands was created by combining only those probands who met criteria for the ADHD-I and ADHD-CO subtypes, and only symptoms of inattention were included in the analyses. In other words, the main paradox this study attempts to address centers on the idea that RD and

| Table 11. Descriptives of Sample - MZ Twins Preferentially Selected for RD |
|---------------|---------------|-------------------|-----------------------|
|               | Affected (RD) | Non-affected      | Affected (RD) v. Non-affected (Independent Samples T-tests; 2-tailed p-values) |
| N             | 267           | 336               |                       |
| Age in years  | 11.51 (2.53)  | 11.96 (2.82)      | Affected > Non-affected, p = 0.042 |
| Parental education | 14.72 (2.16)  | 16.18 (2.19)      | Affected < Non-affected, p = 0.001 |
| Male (%)      | 61.05 (2.16)  | 50.30 (2.19)      |                       |
| Caucasian (%) | 88.62 (2.16)  | 87.82 (2.19)      |                       |
ADHD share genes but yet enter into different types of G x E interactions. Therefore, the hyperactive/impulsive symptom dimension was not included in the analyses since this symptom dimension did not have a genetic correlation with RD. Of note, previous research indicates that the ADHD-primarily inattentive and ADHD-combined subtype probands have similar profiles of neuropsychological impairments (e.g., Chhabildas, Pennington, and Willcutt, 2001). Therefore, there was no reason to suspect that the ADHD-combined subtype probands and the ADHD-primarily inattentive subtype probands would produce different G x E interaction results.

Zygosity of the twin pairs was determined based on selected items from the Nichols and Bilbro (1996) questionnaire. In ambiguous cases, zygosity was determined based on blood sample analyses. Both dizygotic twins were included in this study, and one preferentially selected “affected” monozygotic (MZ) twin from each monozygotic twin pair was included in the study sample if they had at least one sibling who also participated in the study. Both dizygotic twins in a twin pair were included in all analyses with the exception of the environmental main effects and G-E correlation

| Table 12. Descriptives of Sample - MZ Twins Preferentially Selected for ADHD |
|-----------------------------|-----------------------------|-----------------------------|
|                            | Affected (ADHD) | Non-affected | Affected (ADHD) v. Non-affected (Independent Samples T-tests; 2-tailed p-values) |
| N                           | 300             | 662           |                          |
| Age in years                | 11.32(2.71)    | 10.94(2.54)  | n.s.                     |
| Parental Ed.                | 14.92(2.32)    | 14.96(2.55)  | n.s.                     |
| Male (%)                    | 68.0            | 46.7          |                          |
| Caucasian (%)               | 84.6            | 85.9          |                          |
analyses regardless of affection status. All biological parents of twins and siblings included in this study who either completed questionnaire data and/or provided DNA samples were included in this research (see Tables 11 and 12 for demographic variable descriptors of the overall samples including the preferentially selected MZ twins with RD or preferentially selected MZ twins with ADHD).

**DATA REDUCTION AND MISSING DATA**

Some of the variables described above were unavailable for a small subset of children if these measures were not given during the testing battery. Specifically, as mentioned previously, both maternal and paternal education data was not available for some participants. Therefore, when possible, an average of maternal and paternal education values was calculated to create a “Parental Education” variable; however, if parental education data was only available from one parent, only that parent’s data was used. Additionally, the ADHDRS-IV (DuPaul et al., 1998) was administered to as many as three raters for each child. In cases where a fewer number of ratings were available, an average of the available raters was calculated. The distribution for the parental education environmental variable was examined for skewness and kurtosis, and outliers for the parental education variable and for the phenotypic variables were winsorized to within $\pm$ 4 standard deviations from the mean.
**GENOTYPING**

Following informed consent procedures, DNA samples were requested from all participants and their parents. Although blood samples or buccal cell samples were previously collected for a subsample of participants, saliva collection using Oragene kits (DNA Genotek) is currently the primary method of DNA collection. Specifically, participants were asked to donate 2 ml saliva samples into an Oragene sampling kit. Upon closing the sampling vial, the sample is automatically mixed with a preservative. The samples were subsequently mailed to Dr. Shelley D. Smith’s laboratory at the University of Nebraska Medical Center (UNMC) for genotyping.

Once the samples arrived at UNMC, DNA was immediately extracted from the saliva samples following the Oragene manufacturer’s protocol. DNA was then quantified, checked using DNA/RNA spectrophotometric ratios, and genotyped for amelogenin variation to determine that the DNA was of good quality and reflected the gender of the donor. If the aforementioned conditions were not met, a resample was requested immediately. Risk alleles and genotyping method for the ADHD candidate

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Polymorphism</th>
<th>Genotyping Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT1</td>
<td>40-bp VNTR in the 3’ UTR</td>
<td>agarose electrophoresis</td>
</tr>
<tr>
<td>DRD2</td>
<td>TaqI site</td>
<td>agarose electrophoresis</td>
</tr>
<tr>
<td>DRD4</td>
<td>48 bp VNTR in exon 3</td>
<td>agarose electrophoresis</td>
</tr>
<tr>
<td>DRD5</td>
<td>Dinucleotide repeat in the 5’ UTR</td>
<td>automated capillary electrophoresis</td>
</tr>
<tr>
<td>5HTT</td>
<td>44-bp insertion/deletion in promoter region</td>
<td>agarose electrophoresis</td>
</tr>
<tr>
<td>DBH</td>
<td>Dinucleotide repeat 5’ of the transcription site</td>
<td>automated capillary electrophoresis</td>
</tr>
<tr>
<td>ADRA2C</td>
<td>Dinucleotide repeat 6-bp from coding region</td>
<td>automated capillary electrophoresis</td>
</tr>
</tbody>
</table>
Table 14: RD SNPs Genotyped at UNMC

<table>
<thead>
<tr>
<th>Locus</th>
<th>Candidate Genes Investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>15q21:DYX1</td>
<td>DYX1C1</td>
</tr>
<tr>
<td>6p22:DYX2</td>
<td>DCDC2, KIAA0319, TTRAP, THEM2</td>
</tr>
<tr>
<td>3p12: DYX5</td>
<td>ROBO1</td>
</tr>
<tr>
<td>7q35</td>
<td>CNTNAP2</td>
</tr>
<tr>
<td>16q23</td>
<td>CMIP</td>
</tr>
<tr>
<td>16q24.1</td>
<td>ATP2C2</td>
</tr>
</tbody>
</table>

*All locations based on Ensembl release 62, April 2011*

Genes are presented in Table 13. Single nucleotide polymorphism (SNP) data for one marker (rs3758653) of the ADHD candidate gene, DRD4, was also available. Therefore, in addition to the aforementioned DRD4 48 bp VNTR in exon 3 polymorphism, the rs3758653 DRD4 marker in the flanking 5’ UTR region at locus 11p15.5 was also investigated for association and subsequent G x E interactions with RD and ADHD. Data ascertainment and selection methods for rs3758653 DRD4 are described below and were consistent with the ascertainment methods for the RD SNP data.

RD SNP selection (See Table 14) was based on location and minor allele frequency > .10, and the Tagger function in HapMap was used to select the SNPs that best represented the blocks of linkage disequilibrium in the region. Sample sizes for individual analyses will be presented in the Results section below, as sample sizes varied by available genotype and phenotype data.
**PRIMARY ANALYSES**

In what follows, I will describe the rationale behind our choice of association approaches targeting main effects of genotype and environment. Then I will present the G x E methods implemented, including a discussion of power.

**EXAMINING MAIN EFFECTS OF GENOTYPE AND G X E INTERACTIONS**

Although the primary goal of this study is to investigate G x E interactions involving RD and ADHD and parental education, it is important to attempt to replicate those associations between both disorders and environmental and genotypic risk factors that have been documented in the literature. The current research used family based association tests to address main effects of genotype. These analyses were accomplished in the FBAT-GEE procedure (Golden Helix SVS7) with an additive model (Lange, DeMeo, Silverman, Weiss, & Laird, 2004). Replication of association was considered with p values <.05 after Bonferroni correction for multiple comparisons.

The FBAT-GEE procedure (Golden Helix SVS7) which utilizes family-based methods, was employed to analyze genetic main effects on RD and ADHD (Lange et al., 2004). The FBAT approach implements a generalized Transmission Disequilibrium Test (TDT; Spielman, McGinnis, & Ewens, 1993). TDT compare the genotypes of affected

<table>
<thead>
<tr>
<th>Transmitted Alleles</th>
<th>M1</th>
<th>M2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>2n</td>
<td>(2n-w)</td>
<td>2n</td>
</tr>
<tr>
<td>Y</td>
<td>2n</td>
<td>(2n-y)</td>
<td>2n</td>
</tr>
<tr>
<td>Total</td>
<td>w+y</td>
<td>(4n-w-y)</td>
<td>4n</td>
</tr>
</tbody>
</table>

**Table 15: Marker Alleles M1 and M2 Among 2n Transmitted and 2n Nontransmitted Alleles in Families Whose Single Child is Affected**

(Reproduced from Spielman et al., 1993)
individuals to the genotypes of their parents to determine whether a risk allele appears to be transmitted in excess of what would be expected given the laws of Mendelian inheritance. The FBAT program allows for a number of different parent-offspring scenarios to be investigated. In this study, since multiple offspring are phenotyped and genotyped per family, the TDT also accounts for the sibling correlation that contributes to the test statistic generated in FBAT that measures the genetic main effect. Overtransmission of a particular allele from parent to an affected child (e.g., RD or ADHD) provides evidence that the allele is a risk factor for the disease (Table 15). Ultimately, the fbat statistic is calculated as a chi-square of observed versus expected allele distributions, similar to the TDT, in order to test for linkage disequilibrium (see the equation below – with one degree of freedom).

$$\chi^2 = 4n(w-y)^2 / [(w+y)(4n-w-y)]$$

Using causal inference methodology (Pearl, 2000) methods proposed by Vansteelandt et al. (2006), estimates of the effects of the G x E interaction was obtained while taking into account the effects of the genetic main effects. Given the general model provided below, $\beta_1$ is estimate of the genetic main effect, $\beta_2$ is the G x E interaction estimate, $n$ is the number of nuclear families, and $m$ signifies the offspring in the $n$ nuclear families:

$$E(Y_{ij}) = \beta_1^k x(G_{ij}) + \beta_2^k (G_{ij}) z_{ij} + \omega_{ij}(z_{ij}, S_{ij})$$

$$j=1,\ldots,m_i$$
$$i=1,\ldots,n$$
$$k=1,\ldots,p$$
In this model, the $Y_{ij}$ term is the mean-centered phenotype (e.g., RD or ADHD) for the $j$th offspring in the $i$th family. Specifically, an offset value is used to recode the original trait included in the genotype main effects analyses, $Y_{ij}$ ($Y_{ij} = T_{ij} - \mu_{ij}$, where $\mu_{ij}$ is an offset value), and the selection of the offset value increases the power of the FBAT statistic by offsetting the mean of $Y_{ij}$. For dichotomous traits (e.g., RD or ADHD affection status), the literature (Laird et al., 2000; Whittaker and Lewis, 1998) suggests assigning $\mu_{ij}$ as the disease prevalence in the general population. For more common diseases, taking $0 < \mu < 1$ can increase the power of the test (Lange and Laird, 2002), as both unaffected and affected contribute to the test statistic. In this study, very conservative offset values were selected: 0.075 for RD (7.5%; Benton and Pearl, 1978) and an offset of 0.05 was used for ADHD (5%; Satcher, 1999). For continuous phenotypic variables (i.e., inattention or reading), the standard phenotypic residuals were set as the offset value, meaning that the offset equaled the difference between the actual observed phenotype and the predicted phenotype, as calculated by FBAT-GEE (Lange, DeMeo, and Laird, 2002). $X(G_{ij})$ is the RD or ADHD risk alleles for the $j$th offspring in the $i$th family. $Z_{ij}$ is the environmental variable, parental education for the $j$th offspring in the $i$th family. The $k$th component of $\beta_1$ signifies the genetic main effect of the $k$th SNP after taking into account the other SNPs at an identified locus. The $k$th component of $\beta_2$ stands for the effect of the $k$th SNP on the phenotypic trait taking into account the environmental variable. The $\omega_{ij}$ variable represents the variables that are not explicitly stated in the aforementioned model, such as the environmental main effect.
Causal inference methodology utilizes counterfactual theory and graph theory to identify causal and confounding variables (for a more detailed description, see Vansteelandt & Lange, 2006). This methodology allows for valid interpretation of the G x E interaction term after taking to account the genetic main effects included in the model. One of the advantages of using causal inference methodology is that the $\beta_1$ and $\beta_2$ estimates are not dependent on the $\omega_{ij}$ variable, and therefore are valid despite confounds, such as population stratification, which may be present in the data.

Main effects of all candidate genes were investigated for both affection status (i.e., a diagnosis of RD or ADHD) and for the continuous phenotypic variables (i.e., reading or inattention). Since animal literature suggests that G x E interactions occur most often in the presence of main effects, only those alleles that showed genetic main effects on a phenotype were included in subsequent G x E interaction analyses. This step sought to control the Type I error rate by limiting the number of comparisons. Using similar methods described above, the FBAT-GEE procedure (Golden Helix SVS7) FBAT-I function, which allows for the inclusion of an interaction term, was used to test for G x E interactions.

**EXAMINING ENVIRONMENTAL MAIN EFFECTS**

Environmental main effects and gene-environment correlations were assessed using correlational analyses for the continuous phenotypic variables and independent samples t-tests for the analyses involving categorical variables (i.e., affection status).
**Multiple Comparison Correction**

False discovery rate methods (Benjamini & Hochberg, 1995) were used to correct p-values for multiple comparisons after each set of analysis (e.g., ADHD association analyses with ADHD affection status, ADHD association analyses with inattention, RD association analyses with RD affection, etc.).
RESULTS

REPLICATION AND EXTENSION OF ASSOCIATION FINDINGS IN ADHD

We first attempted to replicate association findings from the literature documenting genetic main effects of various ADHD candidate genes and ADHD affection and inattention. Association results, using ADHD microsatellites, revealed non-significant findings for genetic main effects of all variants tested in the current study of DAT1, DRD2, DRD4, 5HTT, DBH, and ADRA2C on both ADHD affection status (affection of ADHD primarily inattentive subtype or ADHD combined subtype) and on the inattentive symptom dimension of ADHD (See Appendix). Although the current study did not replicate findings from Barnard, H.D. (2009) and Bidwell et al. (2011), which showed significant associations between DRD4-4R and ADHD in similar subsets of CLDRC participants, results showed trend level findings of this association (182 families, \( p = .051 \)). Results were non-significant, however, when investigating the

<table>
<thead>
<tr>
<th>Table 16. Association: RD Candidate Genes and ADHD Affection Status</th>
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</thead>
<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>FOXP2</td>
</tr>
<tr>
<td>FOXP2</td>
</tr>
<tr>
<td>KIAA0319</td>
</tr>
<tr>
<td>CNTNAP2</td>
</tr>
</tbody>
</table>
association of the DRD4 rs3758653 SNP and RD or ADHD, which may have been due to reduced power for these analyses due to limited sample sizes.

Next, we sought to replicate and extend the current literature by investigating whether various candidate genes implicated with RD and/or language development (DYX1C1, DCDC2, KIAA0319, TTRAP, THEM2, CMIP, CNTNAP2, ATP2C2, FOXP2) were associated with ADHD (I) affection status and/or inattention using SNP analysis methodology. Results showed non-significant findings for genetic main effects.
of the RD candidate genes tested in the current study and ADHD affection or inattention after correcting for multiple comparisons; however, there was some evidence of genetic main effects of FOXP2 (Figures 3 and 4), KIAA0319 (Figure 5), and CNTNAP2 (Figure 6) on ADHD affection status prior to Bonferroni correction (Table 16).

Given that the above findings were non-significant after multiple comparison correction, we created an additive risk composite to assess whether having a greater number of the aforementioned RD/language risk alleles was associated with ADHD affection status. Individuals were categorized into four groups: those who had between one and two risk alleles (N=94; none of the participants had zero risk alleles), those who had three risk alleles (N=87), those who had four risk alleles (N=80), and those who had five through eight risk alleles (N=92). Groups were created based on sample size, as the number of participants varied at each level of additive risk (e.g., 87 participants had three risk alleles whereas only three participants had eight risk alleles). One-way ANOVA results were non-significant (F = 1.877, p = .133).

Results were also non-significant when exploring the genetic main effects of the RD/language risk genes on the continuous phenotype of ADHD (i.e., the inattentive

| Table 17. Association: RD Candidate Genes and Inattention |
|-------------|---------|----|----------|----------------|-----------------|---------------|----------|--------|
| Gene        | Marker Name | Chromosome | Location | Number of informative families | p-value before correcting for multiple comparisons | Allele Frequency | Parent Allele Frequency | Power-fbat Statistic |
| DCDC2       | rs1340697 | 6     | intron   | 113       | .025              | .698           | .704               | .126               |
| DCDC2       | rs793839  | 6     | intron   | 127       | .023              | .558           | .572               | .051               |
| ROBO1       | rs9871860 | 3     | intron   | 123       | .040              | .674           | .690               | .093               |
symptom dimension) after correcting for multiple comparisons. Prior to multiple comparison correction, however, ROBO1 and DCDC2 showed trend level associations with inattention (see Table 17). Although the DCDC2 rs1340697 marker was associated with inattention before multiple comparison correction, this association appeared spurious upon visual inspection of the data. Specifically, when graphed, this marker did not exhibit the expected pattern showing that an increased number of alleles conferred a greater degree of ADHD inattentive symptomatology. Furthermore, exploratory one-way ANOVA findings were non-significant when investigating whether a greater number of DCDC2 rs1340697 risk alleles conferred more severe ADHD-I symptomatology (F = .787, p = .456).

In addition, KIAA0319 was not associated with the inattentive symptom dimension of ADHD even though it was associated (before Bonferroni correction) with ADHD affection in the current study. Similarly, neither FOXP2 nor CNTNAP2 were associated with the continuous ADHD inattention variable despite their association with ADHD affection. ROBO1 (Figure 7) and DCDC2 (Figure 8), however, showed
associated with the inattentive symptom dimension of ADHD prior to Bonferroni correction but results did not indicate that either of these risk alleles were association with ADHD affection.

Similar to the procedures described above pertaining to the main effects of the RD/language risk genes on ADHD affection status, a composite risk score was created to assess whether the additive effect of the aforementioned DCDC2 loci and the ROBO1 loci had a combined genetic main effect on the inattentive symptom dimension. Participants were grouped into three groups: those who had between zero and two risk alleles (N=123), those who had between three and four risk alleles (N=126), and those who had five or six risk alleles (N=116). One-way ANOVA results did not reveal significant differences between any of the aforementioned groups and severity of ADHD inattentive symptomatology (F = 1.31, p = .271).

Of note, the literature suggests that FOXP2 and CNTNAP2 genes are primarily associated with speech/language disorders (Lai, et al., 2001; Vernes, Newbury, Abrahams, Winchester, Nidoc, Groszer, et al., 2008) but there is little research suggesting pleiotropic effects on ADHD. On the other hand, consistent with the aforementioned trend level findings, although KIAA0319 and DCDC2 have primarily been associated with RD in the literature, recent studies have found evidence for genetic associations with ADHD (Paracchini, et al., 2008; Cuoto et al., 2009).
Using SNP analyses, we then sought to replicate genetic main effects documented in the literature, involving previously identified RD and/or language (DYX1C1, DCDC2, KIAA0319, TTRAP, THEM2, CMIP, CNTNAP2, ATP2C2, FOXP2) language candidate genes and RD. First, we tested for association of the aforementioned risk genes on RD affection status. Results were non-significant for all variants of the aforementioned candidate genes after Bonferroni correction; however, associations with RD affection and DCDC2 and CNTNAP2 were demonstrated prior to multiple comparison correction (Table 18; See Appendix).

Similar to the procedures described above for the ADHD genetic main effects analyses, in order to further test the main effects of the RD/language candidate genes on RD, an additive composite risk score was created to assess whether the DCDC2 and CNTNAP2 loci combined produced a significant genetic main effect on RD affection status. Although findings were in the expected direction, whereby a higher number of RD risk alleles was associated with more RD affection, one-way ANOVA results were non-significant (F = .455 p = .635). Post-hoc comparisons between the three risk groups

| Table 18. Association: RD Candidate Genes and RD Affection Status |
|----------------------|-------------|-------------|-------------|----------------------|-------------|-------------|-------------|---------------|
| Gene                | Marker Name | Chromosome | Location  | Number of informative families | Number of informative families | p-value before correcting for multiple comparisons | p-value before correcting for multiple comparisons | Allele Frequency | Allele Frequency | Allele Frequency | Allele Frequency |
| DCDC2               | rs1340697   | 6           | intron    | 169                                                               | .027                           | .699                         | .704                         | .051            |
| CNTNAP2             | rs7794745   | 7           | intron    | 187                                                               | .042                           | .672                         | .657                         | .054            |

RD risk alleles was associated with more RD affection, one-way ANOVA results were non-significant (F = .455 p = .635). Post-hoc comparisons between the three risk groups
(i.e., those who had zero through two alleles [N=199], those who had three alleles [N=209], those who had four alleles [N=131]) also produced non-significant results.

Consistent with these findings, non-significant results were found when testing the association of the RD/language candidate genes and the continuous reading phenotype after Bonferroni correction. Prior to correction for multiple comparisons, only the DCDC2 (rs793839) allele showed a genetic main effect on reading (182 informative families, p = .042; frequency = .444, parent frequency = .428, power = .159). Taken together, prior to Bonferroni correction, variants of DCDC2 showed association with both RD affection and the continuous RD phenotypic variable as well as the continuous ADHD phenotypic variable, suggesting pleiotropy.

Next, the effects of the ADHD microsatellites (multiple variants of DAT1, DRD2, DRD4, 5HTT, DBH, and ADRA2C) on RD affection were investigated. Non-significant association results were found for all ADHD candidate genes on RD affection status.

When investigating the association of the aforementioned ADHD candidate genes on the continuous word recognition composite variable, results initially seemed to indicate a significant negative association with DAT1-9R (p = .038) before correcting for multiple comparisons. Upon further investigation, however, this potential finding appeared to be spurious, as there were no significant differences in the reading composite scores between participants who had zero, one, or two of the DAT1-9R alleles (one-way ANOVA F = .011 p = .900). Of note, although the DAT1-10R was not significantly associated with the continuous RD phenotypic variable reading before multiple comparison correction, a trend level association was suggested (p = .052). Given this
trend level result coupled with Bidwell and colleagues’ (2011) findings showing a significant genetic main effect of the DAT1-10R allele on the inattentive symptom dimension of ADHD in a similar subsample of CLDRC participants, we conducted an exploratory one-way ANOVA investigating whether individuals with a higher number of DAT1-10R risk alleles had more severe RD symptomatology. One-way ANOVA results were non-significant when comparing groups of participants with zero, one, or two DAT1-10R risk alleles (F = .250, p = .779).

**ENVIRONMENTAL MAIN EFFECTS AND G-E CORRELATIONS**

Although the primary aim of this study was to investigate G x E interaction effects of parental education and RD and ADHD, it was important to replicate the environmental (E) main effect of parental education on RD and ADHD-I symptomatology that have been documented in previous research. Gene-environment (G-E) correlations were not only addressed directly in this study by testing whether there was a relation between the parental education environment and genotype but they were also addressed within the E main effects analyses in order to ensure that those effects attributed to the environment were not actually influenced by genes. The term gene-environment correlation refers to the fact that environments may be partially correlated with genetic factors (Kendler and Baker, 2007; Plomin, 1994; Plomin and Bergeman, 1991; Scarr and McCartney, 1983). Inherently, we expected to find G-E correlations with RD and parental education, as parents with reading difficulties generally have less education and may read less to their children compared to those with typical reading
abilities. Assuming this is the case, parents with reading difficulties are passing down unfavorable alleles for reading while also providing a less enriched literacy environment for their children. Despite this passive G-E correlation, since RD has been shown to follow the bioecological model of G x E interaction, this confound should act as a conservative bias toward our results. In this model, since reading ability is more heritable in a favorable environment, essentially, there will be a negative G-E correlation between environment and offspring RD status. Notwithstanding this conservative bias, delineating the independent influences of the environment compared to those stemming from genetic influences is necessary in order to appropriately interpret E main effects and G x E interactions.

As noted in the Preliminary Studies section, Friend and colleagues (2008) found a significant main effect of parental education on reading ability, using a subsample of CLDRC participants. These findings were replicated in a larger subsample of CLDRC participants (see Preliminary Studies section Tables 3 and 4) showing significant G-E correlations in the expected negative direction for parental education and parent reading and also in the expected negative direction for ADHD-I symptomatology and parental education.

The sample of CLDRC participants included in the current study was more restricted than that referred to in the Preliminary Analyses section, as genotyping data was only available for those identified as meeting inclusionary criteria for either RD or ADHD. Therefore, although there were both affected and non-affected twins in genotyped for this study, twins who were initially recruited as clean controls were not
included in the current study sample. Despite our restricted sample size, we attempted to replicate E main effects and G-E correlations documented in the larger CLDRC samples (see Preliminary Studies).

Of note, as documented in the Procedure section, one MZ twin per twin pair was preferentially selected for RD affection for inclusion in the sample used in the RD analyses and one MZ twin was preferentially selected based on affection status of ADHD for the ADHD analyses. In order to investigate E main effects and G-E correlations, one DZ twin was also randomly selected from each sample so that only one twin per family was included in the analyses, as the parental education environment is typically considered “shared” across children within a family.

Similar to the procedures described above for the Preliminary Studies, we directly tested for E main effects and G-E correlations. Replicating previous findings, independent samples t-tests revealed a significant E main effect of parental education on child reading in the current sample. Bivariate correlations also showed significant G-E correlations in the expected negative direction for parental education and childhood reading when controlling for parent retrospective reports of RD symptomatology (see Table 19), indicating a relation between parent education and child reading apart from the well-established familiality.

<table>
<thead>
<tr>
<th>Table 19. Main Effects of E: Parental Education on Child Reading</th>
<th>N</th>
<th>Pearson’s r</th>
<th>(1-tailed) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bivariate Correlation – Parent Education and Child Reading</td>
<td>147</td>
<td>.280</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Partial Correlation controlling for Parent Reading</td>
<td>48</td>
<td>.288</td>
<td>.021</td>
</tr>
</tbody>
</table>
Next, we investigated whether allele status for those risk alleles that showed trend level genetic main effects was related to parental education. Results revealed non-significant findings for the candidate genes that initially showed trend level genetic main effects with RD affection (DCDC2 rs1340697 and CNTNAP rs7794745) or RD as a continuous phenotypic variable (DCDC2 rs793839), suggesting independent genetic and environmental influences.

Although an E main effect has been previously documented on the larger sample of CLDRC participants (see Preliminary Studies), independent samples t-tests revealed non-significant findings for an E main effect of parental education on ADHD affection (N = 141, t = .674, 2-tailed p = .501) or ADHD-I symptomatology in the current sample (N=141, r = .038, 1-tailed p = .329). Although bivariate correlations controlling for parent retrospective report of ADHD-I symptomatology were tested in order to investigate the relation between parent education and child inattention apart from the familiality of ADHD, the sample size was limited by available parent retrospective self-report data of inattention symptoms (N = 30; r = -.134, p = .463).

Given the documented E main effect of parental education on ADHD-I in the larger overall CLDRC sample, however, we investigated whether allele status for those risk alleles that showed trend level genetic main effects was related to parental education. Results revealed non-significant findings for the candidate genes that showed trend level genetic main effects with ADHD affection (markers rs1270590 and rs2690833 of FOXP2, KIAA0319 rs730860, and CNTNAP2 rs17236239) or the inattentive symptom
dimension of ADHD (DCDC2 rs793839, ROBO1 rs9871860), suggesting independent genetic and environmental influences.

**EXPLORATORY ANALYSIS OF G x E INTERACTIONS**

Since interactions can occur in the absence of main effects, we first investigated the G x E interactions involving all of the RD and ADHD candidate genes with parental education and the continuous and categorical phenotypes of both disorders. Although various significant findings were noted, none remained after multiple comparison correction. Given the high number of comparisons necessary to investigate all of the candidate genes with all of the phenotypic variables, we sought to limit our exploratory analyses to a few select G x E interactions. Specifically, we first attempted to limit our interpretation of the G x E interaction results to those where the candidate gene showed a significant association with the categorical and continuous phenotypic variables (e.g., RD affection and the reading composite continuous phenotypic variable or ADHD affection and inattention) or those that suggested pleiotropic association.

Although none of the specific genetic markers showed main effects (prior to Bonferroni correction) on both the continuous and categorical phenotypes of either disorder (e.g., RD affection status and the word recognition continuous phenotypic variable or ADHD affection status and inattention), results suggested that various markers of DCDC2 were associated with RD affection and reading and also with inattention. Specifically, prior to Bonferroni correction, DCDC2 rs1340697 showed
association with RD affection and inattention; however, the power of the fbat statistic was very low, which may have contributed to the seemingly spurious findings with inattention. DCDC2 rs793839 also showed association with the continuous phenotypic variables of RD and ADHD. Although the fbat statistical power was low for association with inattention, it was slightly higher with reading. Taken together, given the potential suggestion of pleiotropy, we conducted exploratory G x E interaction analyses involving DCDC2 and parental education on RD or ADHD. Results were non-significant for G x E interactions involving DCDC2 and parental education on either inattention or ADHD affection. A trend level G x E interaction finding was found when testing the G x E interaction with DCDC2 and RD affection for the rs9460974 marker, however, this finding did not remain after multiple comparison correction (see Table 20). Consistent with these non-significant results, a clear G x E interaction pattern (i.e., bioecological or diathesis-stress) involving either the continuous or categorical phenotypic variables of RD or ADHD was not evident upon visual inspection of these data.

Next, our exploratory G x E interaction analyses focused on those candidate genes that either showed a suggestion of pleiotropic effects in the current sample (even if the candidate genes were not associated with both the categorical and continuous phenotypic

| Table 20. G x E Interactions:DCDC2 rs9460974 with Parental Education and RD or ADHD |
|-----------------------------------------------|---------------------------------|---------------------|
| Informative Families | fbat-I p-value (combined main effect and interaction term) | Power - fbat-I Statistic |
| RD Affection | 127 | .050 | .05 |
| RD Continuous | 127 | .078 | .24 |
| ADHD Affection | 89 | .630 | .05 |
| ADHD Continuous | 89 | .702 | .05 |
variables for RD or ADHD) or those in which pleiotropy has previously been documented in the literature. Specifically, in addition to DCDC2, previous studies have shown pleiotropic effects of KIAA0319 (e.g., Paracchini et al., 2008; Cuoto et al., 2009). Therefore, we investigated the G x E interactions involving KIAA0319 and parental education on RD or ADHD. Although KIAA0319 only showed a genetic main effect with ADHD affection, there was evidence for G x E interactions between KIAA0319 and parental education on both ADHD affection and the inattentive symptom dimension prior to Bonferroni correction. Results were non-significant for KIAA0319 rs730860 and the RD phenotypes (see Table 21).

A median split was applied to the parental education variable in order to investigate the direction of the G x E interaction of the KIAA0319 rs730860 marker with parental education on ADHD (I) affection status and also on the inattentive symptom dimension.

### Table 21. G x E interaction: KIAA0319 rs730860 with Parental Education and RD or ADHD

<table>
<thead>
<tr>
<th></th>
<th>Informative Families</th>
<th>fbat-I p-value (combined main effect and interaction term)</th>
<th>Power- fbat-I Statistic</th>
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</thead>
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<tr>
<td>RD Affection</td>
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<td>.924</td>
<td>.151</td>
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<td>RD Continuous</td>
<td>138</td>
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<td>.508</td>
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<tr>
<td>ADHD Affection</td>
<td>93</td>
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<td>.344</td>
</tr>
<tr>
<td>ADHD Continuous</td>
<td>93</td>
<td>.040</td>
<td>.164</td>
</tr>
</tbody>
</table>

Figure 9.
symptom dimension. Although G x E interaction findings were non-significant after Bonferroni correction, results suggested a bioecological G x E interaction for both the categorical and continuous phenotypic variables of ADHD (See Figures 9 and 10), whereby KIAA0319 rs730860 was preferentially over-transmitted to children with ADHD (I) in the high parental education environment compared to the low parental education environment. It is important to note, however, that the sample sizes were very small for the individuals with two risk alleles in the high and low parental education environments. Specifically, Figure 9 depicts an N of five individuals in the high parental education group who had two risk alleles: five affected individuals and zero unaffected individuals. Similarly, Figure 9 depicts five individuals in the low parental education group with two risk alleles: two affected individuals and three unaffected individuals. In Figure 9, a total of 19 individuals in the high parental education group with one risk allele (7 affected, 12 unaffected) and 17 individuals in the low parental education group with one risk allele (7 affected, 10 unaffected) are depicted. Finally, 31 individuals in the high parental education group with zero risk alleles (4 affected, 27 unaffected) and 25 individuals in the low parental education group with zero risk alleles (7 affected, 18 unaffected) are
shown. Of note, fbat imputes missing genetic data if enough information is available from other family members’ genotypes. Therefore, two families are not depicted in Figures 9 and 10 although they are included in the fbat G x E interaction analyses for KIAA0319, parental education, and ADHD-I affection.

Not surprisingly, given the non-significant genetic main effects findings for the RD phenotypic variables, G x E interaction results did not show a clear pattern involving KIAA0319 rs730860, parental education, and either the categorical or continuous RD phenotypic variables.

Additionally, since CNTNAP2 rs17236239 showed association with ADHD affection status, and CNTNAP2 rs7794745 showed association with RD affection status before correcting for multiple comparisons, we investigated the G x E interactions involving CNTNAP2, parental education, and RD or ADHD. Results suggested a significant G x E interaction with CNTNAP2 rs17236239 and parental education on inattention; however, upon closer visual analysis of these data, a clear G x E interaction was not evident. Results were non-significant for the G x E interactions involving CNTNAP2 rs17236239 and parent education on ADHD affection or the RD phenotypic variables (see Table 22).

<table>
<thead>
<tr>
<th>Table 22. G x E interactions: CNTNAP2 rs17236239 with Parental Education and RD or ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informative Families</td>
</tr>
<tr>
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</tr>
<tr>
<td>RD Continuous</td>
</tr>
<tr>
<td>ADHD Affection</td>
</tr>
<tr>
<td>ADHD Continuous</td>
</tr>
</tbody>
</table>
Power calculations were implemented in the FBAT-GEE for affection status and for the continuous trait association analyses. Although power cannot be calculated for the interaction term specifically (FBAT-I), FBAT analyses that include an interaction term produce omnibus values that incorporate both main effects and interactions. Power calculations for the fbat and fbat-I (i.e., the power of the interaction and the genetic main effect) statistics are documented above in the corresponding sections of the Results.
DISCUSSION

The primary aim of this study was to advance the understanding of the multifactorial etiology of RD and ADHD by examining the reasons for opposite G x E interactions influencing each disorder (i.e., bioecological for RD and diathesis-stress for ADHD) using molecular genetics methods. Specifically, the goals of this study were to 1.) partition known risk alleles for RD and/or language development (DYX1C1, DCDC2, KIAA0319, TTRAP, THEM2, CMIP, CNTNAP2, ATP2C2, FOXP2) and ADHD (DAT1, DRD2, DRD4, 5HTT, DBH, and ADRA2C) into those that are pleiotropic and those that are non-pleiotropic using family based association methods, 2.) replicate and extend the current literature on the G x E interactions influencing RD and ADHD by exploring the G x E interactions involving RD and ADHD candidate genes and parental education, and 3.) if opposite G x E interactions were found in the expected directions (e.g., bioecological for RD risk genes and parental education and diathesis-stress for ADHD risk genes and parental education), test two competing hypotheses to further investigate the paradox described above: a.) the opposite G x E interactions are due to non-pleiotropic risk alleles and not to pleiotropic ones and b.) the opposite G x E interactions are due to differing proximal environmental variables, such as parent literacy practices, that are nested under the broad environmental variable, parental education.
OVERALL SUMMARY OF FINDINGS

Although the primary goal of this study was to further explore the opposite G x E interactions influencing RD and ADHD, we first investigated the genetic main effects of the aforementioned RD and ADHD candidate genes on each disorder. The most compelling trend level main effect was that of DCDC2 on RD as DCDC2 was associated with both the categorical (i.e., RD affection status) and continuous (i.e., reading) phenotypic variables of RD. Although the other candidate genes did not show association with both the categorical and continuous phenotypic variables, findings suggested a fair amount of pleiotropy between previously identified RD or language-related candidate genes and those associated with ADHD. Specifically, results showed potential main effects of DCDC2 and CNTNAP2 on both RD and ADHD. In addition, findings suggested potential main effects of previously identified RD or language-related candidate genes, KIAA0319, ROBO1, and FOXP2, on ADHD.

Despite non-significant association findings after correcting for multiple comparisons, the pattern of results from this study suggested pleiotropy with KIAA0319 and DCDC2, which is consistent with current literature (Walcott et al., 2002; Paracchini et al., 2008; Cuoto et al., 2009; Barrett, Fry, Maller). Interestingly, as Cuoto and colleagues (2009) indicate, although various studies have either shown genetic main effects of DCDC2 or KIAA0319 on RD (e.g., Schumacher et al., 2006; Ludwig et al., 2008; Wilcke et al., 2009) or ADHD (e.g., Willcutt et al., 2002; Cuoto et al., 2009), there are no studies to date showing robust associations of DCDC2 on RD and ADHD within
the same sample. Although results from the current study documented associations between various markers of DCDC2 on RD and ADHD, these findings did not remain significant after multiple comparison correction. Therefore, further investigation into the pleiotropic effects of DCDC2 on RD and ADHD in larger samples with more power to detect genetic association is necessary. In addition, consistent with previous research, we found a trend level association of KIAA0319 with ADHD but not with RD in the current study sample.

Moreover, we found associations for CNTNAP2 and both RD and ADHD affection, between two markers of FOXP2 and ADHD affection, and between one marker of ROBO1 and inattention before correcting for multiple comparisons. Although findings did not remain significant after Bonferroni correction, results suggested trend level pleiotropic effects for the aforementioned RD/language candidate genes.

To date, there has been little research documenting the pleiotropic effects of previously identified language-related candidate genes, CNTNAP2 (7q35), FOXP2 (7q31), and ROBO1 (3p12) and ADHD (Elia et al., 2010). Interestingly, CNTNAP2 is a gene regulated by FOXP2 and has shown high expression in anterior frontal circuits (Vernes et al., 2008), and FOXP2 has been associated with striatal abnormalities (Watkins et al., 2003; Takahashi et al., 2003). Although both CNTNAP2 and FOXP2 have been primarily associated with language development in the literature (e.g., specific language impairment), it is not surprising that these genes may also be associated with ADHD, as disruptions to cortico-striatal pathways, particularly pathways extending from the prefrontal cortex to the striatum, have been shown in ADHD (e.g., Castellanos, 2001).
In addition, since ROBO1 has been shown to play a role in axonal growth and migration (Hannula-Jouppi et al., 2005), it would not be surprising to find pleiotropic findings involving multiple neurodevelopmental disorders. Despite the trend level findings in the current study, further replication with larger samples is necessary to delineate the suggested pleiotropic effects of CNTNAP2, FOXP2, and ROBO1.

Replicating previous findings (Friend et al., 2010), analyses also indicated a significant environmental main effect of parental education on child reading even when controlling for the potential gene-environment (G-E) correlation by partialling out parent retrospective reading symptoms. An environmental main effect of parental education on childhood ADHD-I was not found when including only those participants who had available genetic data; however, this relation was previously documented in the larger overall CLDRC sample. Therefore, non-significant E main effect findings in the current sample are likely due to limited sample size and reduced power. Results did not reveal significant G-E correlations of the parental education environment and genotype for any of the candidate genes considered for the exploratory G x E interaction analyses.

As noted above, although significant findings did not remain for any of the genetic main effects analyses after Bonferroni correction, we conducted exploratory G x E interaction analyses involving CNTNAP2, KIAA0319, and DCDC2 with parental education and the ADHD and RD phenotypic variables. Similar to the genetic main effects analyses, all findings were non-significant after correcting for multiple comparisons. Interestingly, prior to Bonferroni correction, initial analyses suggested a bioecological G x E interaction involving KIAA0319 rs730860 and parental education on
both RD affection and inattention, whereby KIAA0319 rs730860 was preferentially transmitted to children with ADHD in the high parental education environment compared to the low parental education environment.

Since we did not find the opposite G x E interactions documented in the literature (i.e., bioecological for RD, diathesis-stress for ADHD), we were unable to test the two competing hypotheses: 1.) that the opposite G x E interactions documented in the literature are due to non-pleiotropic risk genes not to pleiotropic ones, and 2.) the opposite G x E interactions are due to differing proximal environmental measures, such as parent literacy environment. Although we did not find the expected opposite G x E interactions for RD and ADHD, the aforementioned G x E interaction findings, suggesting a bioecological model of interaction involving KIAA0319, parental education, and ADHD, provided some tentative support for the genetic hypothesis, as KIAA0319 has been primarily associated with RD in the literature. On the other hand, research suggests that KIAA0319 has pleiotropic effects on both RD and ADHD, and the genetic hypothesis indicates that the pleiotropic genes do not drive the opposite G x E interactions observed in both disorders. Moreover, although KIAA0319 has primarily been associated with RD in the literature, results found non-significant genetic main effects on RD in the current study and only suggested a trend level main effect with ADHD. Consistent with these findings, robust associations with both RD and ADHD have not been documented in the same samples in the current literature. Therefore, although the aforementioned G x E interaction results indicating a bioecological model of interaction involving KIAA0319, parental education, and ADHD (affection and
inattention) are compelling, replicating these results using larger samples is necessary. Furthermore, it will be important to replicate the G x E interaction effects in studies that demonstrate pleiotropy within the same sample.

CONCLUSIONS

Taken together, this study suggests two conclusions. First, these findings suggest a fair amount of pleiotropy within candidate genes previously identified for RD and/or language development and those candidate genes previously identified for ADHD. Second, replicating previous findings, this study supports the idea that many of the pleiotropic genes that influence the manifestation of RD or language phenotypes and ADHD are those that have been shown to play a role in neurodevelopmental processes, such as neuronal migration and axonal growth and migration.

Importantly, this was the first study to explore G x E interactions for RD and ADHD using molecular genetics methods in the same overall sample. Although none of the findings in the current study remained significant after multiple comparison correction, exploratory analyses showed trend level G x E interactions involving KIAA0319, parental education, and ADHD affection and inattention. Overall, this study highlights the complex, multifactorial etiologies of RD and ADHD; however, replication and extension of these findings is necessary.
LIMITATIONS AND FUTURE DIRECTIONS

First, although preliminary power analyses were investigated prior to commencing this study, results demonstrated that these analyses were underpowered. Given the various stages of the data collection and processing, sample sizes varied by candidate gene and by phenotype. Therefore, it will be important for future studies to utilize larger samples to ensure adequate power to detect genetic and environmental main effects. This issue is especially relevant for the exploratory G x E interaction analyses, as inclusion of an interaction term reduces power. In addition, the current study included a broad range of previously identified risk alleles for both RD and ADHD. In order to reduce the number of multiple comparisons, future studies may wish to narrow the scope of the analyses (e.g., only include those markers that showed trend level association in the current study or that have shown replication previously in the literature).

Furthermore, it is possible that due to the selection procedures and demographics in this study, the parental education environmental variable included in the G x E interaction analyses may have been too restricted to detect significant interactions. In other words, it is possible that the range of the parental education variable used to define the “risk” and “non-risk” parental education environments was not disparate enough to detect significant interactions. In addition, the participants in the study were volunteers recruited from mainly suburban Denver populations, and therefore, these findings might not generalize to more diverse populations. Given these limitations, it will be important for future studies to ensure an adequate range of environmental variables, as restricted range can influence G x E interaction results. Furthermore, it will be important to
investigate the pleiotropic effects of both RD and ADHD candidate genes and replicate pleiotropic findings that have been documented in different samples within the same sample.

Moreover, it is likely that there are additional genetic risk factors (not under consideration in this study) that may interact with the targeted risk alleles for RD and/or ADHD to produce G x G interactions, in addition to the G x E interactions, which influence RD and ADHD manifestation. In addition to G x E interactions, future studies may choose to explore the effects of specific G x G interactions on RD and ADHD.


Castellanos (Eds.), *Stimulant drugs and ADHD: Basic and clinical neuroscience* (pp. 243-258). Oxford: Oxford University Press.


Elia, J., Gai, X., Xie, H.M., Perin, J.C., Geiger, E., Glessner, J.T., et al. (2010). Rare structural variants found in attention-deficit hyperactivity disorder are preferentially associated with neurodevelopmental genes. Mol Psychiatry, 15, 637-646.


International Dyslexia Association Board of Directors [IDA]. November 12, 2002.


## Association Results: Raw p-values Before Multiple Comparison Correction

<p>| Genetic Markers | Phenotypes | Phenotypes |  |  |
|-----------------|------------|------------|  |  |
|                 | RD Affection | RD Continuous | ADHD-I Affection | ADHD-I Continuous |
| <strong>ROBO1</strong>       |             |             |  |  |
| rs9837496       | 0.074       | 0.627       | 0.932 | 0.366 |
| rs9871860       | 0.447       | 0.638       | 0.965 | <strong>0.036</strong> |
| rs328045        | 0.741       | 0.801       | 0.162 | 0.771 |
| rs10514741      | 0.976       | 0.751       | 0.051 | 0.236 |
| rs333493        | 0.902       | 0.921       | 0.803 | 0.245 |
| rs7640444       | 0.322       | 0.415       | 0.739 | 0.764 |
| rs17397244      | 0.199       | 0.403       | 0.630 | 0.859 |
| <strong>DCDC2</strong>       |             |             |  |  |
| rs2274305       | 0.733       | 0.128       | 0.405 | 0.129 |
| rs1419228       | 0.051       | 0.608       | 0.553 | 0.439 |
| rs793862        | 0.645       | 0.119       | 0.535 | 0.172 |
| rs2328208       | 0.373       | 0.190       | 0.666 | 0.289 |
| rs807701        | 0.582       | 0.108       | 0.253 | 0.104 |
| rs2753912       | 0.474       | 0.529       | 0.542 | 0.079 |
| rs2791972       | 0.816       | 0.214       | 0.870 | 0.472 |
| rs793663        | 0.410       | 0.499       | 0.203 | 0.187 |
| rs4607425       | 0.948       | 0.742       | 0.189 | 0.214 |
| rs9460974       | 0.136       | 0.824       | 0.875 | 0.781 |
| rs1340697       | <strong>0.027</strong>   | <strong>0.073</strong>   | <strong>0.386</strong> | <strong>0.025</strong> |
| rs6456605       | 0.918       | 0.617       | 0.223 | 0.887 |
| rs793839        | <strong>0.120</strong>   | <strong>0.042</strong>   | <strong>0.200</strong> | <strong>0.023</strong> |
| <strong>KIAA0319</strong>    |             |             |  |  |
| rs6902039       | 0.948       | 0.521       | 0.335 | 0.299 |
| rs3756821       | 0.496       | 0.070       | 0.441 | 0.439 |
| rs807509        | 0.287       | 0.605       | 0.828 | 0.945 |
| rs2760179       | 0.516       | 0.910       | 0.715 | 0.407 |
| rs2817201       | 0.560       | 0.557       | 0.426 | 0.338 |
| rs9295626       | 0.950       | 0.740       | 0.537 | 0.548 |
| rs7755563       | 0.357       | 0.539       | 0.673 | 0.733 |
| rs761100        | 0.549       | 0.882       | 0.286 | 0.656 |
| rs730860        | <strong>0.958</strong>   | <strong>0.104</strong>   | <strong>0.039</strong> | <strong>0.125</strong> |
| rs12196913      | 0.323       | 0.066       | 0.271 | 0.433 |</p>
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<th>6R</th>
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**DRD4**

| rs3758653 | 0.808 | 0.401 | 0.760 | 0.526 |
| 7R | 0.834 | 0.445 | 0.138 | 0.274 |
| 5R | N/A | N/A | N/A | N/A |
| 4R | 0.623 | 0.656 | 0.051 | 0.095 |
| 3R | 0.762 | 0.500 | 0.519 | 0.861 |
| 2R | 0.093 | 0.641 | 0.959 | 0.677 |

**DRD2**

| 2R | 0.270 | 0.269 | 0.465 | 0.602 |
| 1R | 0.270 | 0.269 | 0.465 | 0.602 |

**DBH**

| 5R | 0.441 | 0.417 | 0.896 | 0.637 |
| 4R | 0.358 | 0.398 | 0.203 | 0.967 |
| 3R | 0.907 | 0.852 | 0.307 | 0.723 |

**DAT1**

| 10R | 0.651 | 0.052 | 0.848 | 0.817 |
| 9R | 0.522 | 0.038 | 0.749 | 0.730 |

**ADRA2C**

| 7R | 0.953 | 0.502 | 0.884 | 0.351 |
| 6R | 0.954 | 0.504 | 0.733 | 0.625 |

**5HTT**

<p>| 2R | 0.332 | 0.807 | 0.546 | 0.536 |</p>
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<th>1R</th>
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* Rows highlighted in yellow indicate candidate genes that showed at least trend level association with both the affection and continuous variables for a phenotype (i.e., RD affection and RD continuous or ADHD affection and ADHD continuous). Bolded cells indicate significance before multiple comparison correction.